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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Clinical Trial Designs for Advanced Therapies

Guest Edited by Dr Timothy Miller

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ISSUE SPOTLIGHT:

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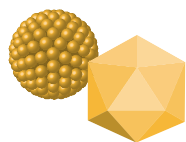

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EXPERT INSIGHT

Towards individualized, low toxic conditioning in autologous gene-transduced hematopoietic cell transplantation

Rick Admiraal, Susan Prockop & Jaap Jan Boelens

Allogeneic hematopoietic cell transplantation (Allo-HCT) has become much safer over the last couple of years. This, together with the rapidly evolving autologous HCT-gene therapy options, is expected to result in an increase in the number of patients receiving HCT. Autologous HCT-gene therapy is a more advanced, safer and precise option for monogenetic life threatening disorders. The efficacy of gene therapy, however, does not only rely on the gene construct itself, but also on the conditioning applied before the gene therapy. In this review we describe how the conditioning can impact the outcomes of the allo-HCT and gene therapy and we will provide a future perspective on how to further improve the efficacy and reduce the short- and long-term toxicity of the conditioning.

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INTRODUCTION

In pediatric allogeneic hematopoietic cell transplantation (allo-HCT) there is a long-standing history of performing transplants for malignant and non-malignant disorders. Most of the non-malignant diseases are mono-genetic diseases of the immune-system, red cells,

lysosomal enzyme deficiencies and bone marrow failure syndromes. As transplant has become safer over the last decade, the number of patients receiving an allogeneic-hematopoietic cell transplantation (allo-HCT) for these disorders has increased to approximately 50% of the pediatric patients transplanted annually worldwide (CIBMTR.org). With safer allo-HCT strategies and the rapid evolving autologous HCT-gene-therapy options, the expectation is that this number will further grow in upcoming decade. Also, with the advent of new technologies, it is expected that standard allo-HCT transplants to be gradually replaced by autologous gene-transduced hematopoietic cell transplantation (gene-therapy: GT) for patients with a mono-genetic life-threatening disorder: a more advanced, safer, precision strategy. The effect of the GT does however not only rely on the gene-construct itself but also on the conditioning applied before the GT. In other words, the efficacy of a GT treatment depends on the package of conditioning and GT product combined. In this review we describe how the conditioning can impact the outcomes of the allo-HCT and GT and we will provide a future perspective on who to further improve the efficacy and reduce the short- and long-term toxicity of the conditioning.

BACKGROUND

It is well recognized that differences in conditioning regimen may contribute to differences in outcome [1]. Even within patients receiving the same conditioning regimen with comparable doses, outcomes may not be the same, due to variability in pharmacokinetics (PK) and pharmacodynamics (PD) of agents used in the conditioning [2–4]. PK includes all processes that influence concentration over time, i.e., clearance, distribution, absorption, etc. PD on the other hand describes the relationship between concentration over time and drug effects (efficacy and toxicity). Variables such age, body size, organ function and concomitant medications can influence the PK profile resulting in variable PD outcomes. Busulfan, an alkylating agent, is the best studied agent in the conditioning regimen and seems also crucial in the conditioning before GT [5–11]. Therefore we will mainly focus on this agent, because immune suppression with agents like fludarabine and ATG (anti-thymocyte globuline) are less/not relevant in the conditioning for GT. Busulfan PK has been studied by several groups (adult and pediatric), which has led to several PK-models, mainly developed in cohorts of infants and children but also in some adult cohorts [4,5,7]. The optimal therapeutic window of busulfan exposure has been established in multiple reports [5,8]. This optimal exposure appears to be independent on cell source, match grade, indication and concomitant conditioning agents. Although the optimal exposure was similar when receiving 1 (Busulfan as single alkylator), 2 (Busulfan combined with cyclophosphamide; Cy) or 3 alkylators (Busulfan, Cy and Melphalan), patients receiving only busulfan combined with fludarabine had lowest toxicity and superior overall survival chances (due to lower toxicity: e.g., veno-occlusive disease [VOD], graft versus host disease [GvHD] and idiopatic pneumonia syndrome

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[IPS]). The optimal cumulative target exposure for Bu AUC0–4 days was found to be 90 mgxh/L (range 80–100 mg*h/L, over 4 days), for all cell sources, including cord blood [5]. Optimal myeloablation also seems of great importance for GT. Sessa *et al.* showed in the lentiviral GT trial in early-onset metachromatic leukodystrophy that patients with sub-ablative exposure of busulfan had lower engraftment of gene-transduced cells, resulting in lower (not supra-normal; which was the goal of this intervention) enzyme levels [12]. Patients with ablative (AUC0-4 days of 80–100 mg*h/L) busulfan exposure achieved supra-normal levels (5–10 times normal) [12]. Also, in other autologous gene-transduced HCTs, aka GT (e.g., Wiskot Aldrich, Fabry disease, Beta-thalassemia) sufficient ablation seems important for optimal effect.

POPULATION PHARMACOKINETICS & PHARMACODYNAMICS: TOWARDS PRECISION DOSING IN TRANSPLANT

Because the GT solutions developed are for congenital diseases, most GT transplanted patients will be pediatric patients, although some congenital disease may occur in adulthood. Knowing this we need to understand that many drugs (used in transplant) are not evaluated in children, contributing to off-label or unlicensed use in as high as 49–87% of drugs used in tertiary care hospitals [13]. Pediatric dosing regimens are often empirical, linearly extrapolated from adult dosing based on body weight. When using a per kilogram dose, the assumption is made that the pharmacokinetics (PK; e.g., clearance, volume of distribution) also increase linearly with body weight in order to reach comparable concentrations. In addition, the assumption is made that the concentration–effect relationship is comparable between children and adults. However, since developmental changes are mostly non-linear [14], empirical dosing can lead to underdosing or overdosing. This is especially true in the very young children and adolescents, thereby introducing toxicity or reduced efficacy [15,16]. In order to reach optimal exposure in all patients, the PK and pharmacodynamics (PD) need to be described, including the influence of predictors such as body size on PK and PD. With these models, the optimal dose for any individual patient can be predicted to reach optimal exposure. This approach has been demonstrated in pediatric HCT [10]. While most cytostatic agents used in HCT are dosed using a fixed mg/kg or mg/m^2 dose for all patients, busulfan dose is fully individualized and controlled using therapeutic drug monitoring (TDM) [10]. Recent work has shown that actual exposure to busulfan impacts outcome in terms of toxicity, graft failure and relapse as described above [4,5].

The population approach, using advanced non-linear mixed effects modeling and high computing power, is the preferred method for PK analyses according to both the FDA and EMEA guidelines [17,18]. In the population approach, data from all patients is pooled to estimate a population mean for all PK-parameters [19]. Next, based on individual concentrations inter-individual variability and residual error are calculated for each patient.

Main advantage of the population approach is the ability to use sparsely sampled and unbalanced (differences in number of samples and sample times between patients, as often the case) data [20]. This makes the population approach particularly attractive in pediatrics, where few samples are available, and the absolute dose varies significantly between children. Additionally, the estimation of PK-parameters is more robust compared to non-compartmental analyses such as the two-stage approach as the software is able to differentiate between real inter-individual variability and residual error (a combination of incorrect sample times, measurement errors and model misspecification) [21]. Altogether, from an ethical, practical and methodological point of view, the population approach is the preferred method for PK analyses.

After describing the population pharmacokinetics, the relationship between concentrations or exposure and effects or toxicity (PD) needs to be determined. The PD-analysis will give further insight into the therapeutic window and will set an optimal target exposure. Next, an individualized dosing regimen can be designed using the population PK model, aiming for optimal exposure. The proposed individualized dosing regimen should be evaluated in a prospective trial, both for external validation of the PK-model and the clinical safety and efficacy [2].

Individualized drug dosing is increasingly incorporated, especially in pediatrics where differences in PK between children of different age groups are major. While individualized dosing regimens are designed according to the above in many fields, we feel HCT is at the front of incorporating the individualized dosing in clinical practice. As such, most centers use individualized busulfan dosing with therapeutic drug monitoring [5,10]. Less of interest for conditioning in GT (more for allo-HCT), individualized dosing for ATG and fludarabine has been designed and is currently evaluated in clinical trials based PKPD analyses [22–24]. PKPD analyses is also of importance for novel, chemo-free regimens in the future, such as described in next section.

Finally, the currently available models may be further sophisticated, describing not only PK or PD, but rather the complete spectrum of drug treatment, including dose, PK, biomarker response, clinical efficacy and toxicity in one comprehensive model. We expect development and implementation of individualized dosing to take place in the next 10 years, thereby improving the knowledge and efficacy of clinical drug therapy, and improving clinical outcome following HCT. With individualized dosing, unwanted variability in drug exposure will be reduced, leading to predictable, adjustable and improved outcome of allo-HCT and GT.

ANTIBODY-BASED CONDITIONING AS FUTURE PERSPECTIVE

In addition to achieving better efficacy and less toxicity by improving the way agents are dosed in conditioning regimens for allo-HCT and GT, there is an emerging approach exploring the use of antibody-based conditioning. This because chemotherapy-based regimens, although due to PKPD considered less toxic in the short term, they come with

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significant late effects, including infertility. Antibody based conditioning relies on using specifically targeted antibodies to transiently or permanently deplete components of the recipient hematopoietic system. There is the potential to use naked antibodies or antibodies conjugated to drug or radiolabeled. In addition, this approach could be used in isolation or in combination with chemotherapy.

Early trials (NCT00590460, NCT00056979 and NCT00579137) run by investigators at Baylor College of Medicine and Texas Children's used a combination of reduced intensity chemotherapy (fludarabine and low dose irradiation or cyclophosphamide) with monoclonal antibodies targeting most hematopoietic cells (anti-CD45 antibodies YTH.24 and YTH.54) in combination with alemtuzumab (anti-CD52). As reported in ClinicalTrials.gov a total of 15 patients have received this antibody combination including three patients with SCID and five with Fanconi anemia. Published results are reported in two patients with SCID [25] one of whom engrafted, and in seven patients transplanted for malignant disease who had comorbidities limiting the use of more aggressive cytoreduction [26]. These seven were transplanted from unrelated donors after cytoreduction with fludarabine, 450cGy of TBI, alemtuzumab and CD45 monoclonal antibodies [26]. Six of these seven recipients engrafted including 3 of 4 recipients of mismatched unrelated grafts. In this study the dosing of the anti-CD45 monoclonal antibodies was based on kinetics previously established in a rat model [27]. In addition, as these patients received T-replete stem cell grafts, engraftment may have been mediated by donor immune populations not only antibody mediated immune ablation. While there is preclinical data suggesting it might be possible [28] it remains controversial whether anti-CD45 targeting antibodies alone can effectively achieve conditioning for transplant [29]. Methods to enhance the efficacy of anti-CD45 are being explored including radio-conjugated Anti-CD45 (I131) Apamistamab [30] or drug conjugated anti-CD45 with saponin or Amanitin [31,32].

Depletion of recipient hematopoietic stem cells (HSC) alone may be sufficient to achieve clinically meaningful responses in the setting of autologous GT for diseases where partial engraftment of genetically modified HSCs is sufficient to correct the phenotype or in allo-HCT for disorders of immunity where the donor cells cannot be rejected and partial donor chimerism is sufficient to correct the phenotype. The potential for antibody mediated depletion of hematopoietic stem cells has been demonstrated using the monoclonal antibody ACK2 in an immune deficient mouse model [33] and in a Fanconi anemia mouse [34]. ACK2 recognizes and antagonizes c-kit and interferes with the interaction between c-kit and its ligand stem cell factor [35]. Administration of the monoclonal antibody resulted in transient decreases in phenotypic and functional HSCs in the recipient mice. In addition, although the decrease was transient, infusion of purified donor HSCs during a window after clearance of the antibody from the serum, but prior to recovery of endogenous HSC function allowed for durable donor chimerism. A humanized version of this antibody, AMG 191, is now in clinical

trial (NCT02963064) with promising early results presented in abstract at TCT 2019 [36]. This trial employs antibody depletion to achieve stem cell engraftment for individuals with SCID who despite prior transplant do not have donor myeloid engraftment and have poor immunity. As demonstrated in the-preclinical studies [37], the timing of infusion of donor HSCs relative to antibody level is critical to engraftment. The ongoing clinical trial is a dose escalation trial that involves real time monitoring of serum antibody level to determine individualized timing for infusion of the stem cell graft.

The HSC clearance mediated by AMG 191 is in part dependent on Fc-mediated effector functions [38] which may restrict its applicability in patients with defective effector function. In addition, to extend this approach to settings where high level chimerism is required, the use of a combination of anti-c-kit and anti CD47 [38] to enhance the activity of anti-c-kit depletion by blocking the CD47-SIRPa interaction and enhancing phagocytosis is being developed. In addition, as in the case for anti-CD45, pre-clinical data is emerging for the use of saponin and amanitin conjugated CD117 antibodies [39–41].

Additionally, there are examples where antibody mediated lymphoid depletion alone may be sufficient such as lymphodepletion prior to adoptive therapy with CAR T cells. Similarly, to the timing of infusion of hematopoietic stem cells after stem cell targeting antibody, the timing of infusion of CAR T cells would depend on clearance of T cell targeting antibody(ies) used for depletion.

In some instances, where complete donor HSC engraftment and immuno-ablation are required, the combination of antibody-based conditioning with conventional reduced intensity chemotherapy may be necessary. These approaches will all likely require individualized (PK guided) dosing as for example, combining conventional chemotherapy with antibody based therapy can change the kinetics of clearance and the biodistribution of the antibody [42].

Understanding the kinetics of monoclonal antibody clearance and individualized dosing will be critical to the success of these trials. Effective use of antibody-based depletion may require ongoing TDM as the kinetics of effect and of clearance will likely depend on non-linear factors such as the size of the target population of cells. Thus, as in the example provided by Busulfan, careful monitoring of dosing kinetics and individualized timing of dosing if not dosing itself, will likely be essential to the broader application of these approaches that have the potential to decrease the toxicity of our therapies.

IN CONCLUSION

Allo-HCT has become much safer over the last couple of years. An important factor in this is applying knowledge gathered from PK and PD analyses of agents used in the conditioning regimens. Individualizing agents used in the conditioning regimens prior to allo-HCT is also important for GT to achieve sufficiently high engraftment of gene-transduced cells to prevent disease. Currently busulfan (with an optimal myelo-ablative target of 90; 80–100 mg*h/L) seems the best studied and easiest to

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target myeloablative agent used in allo-HCT and GT. Although short-term toxicities if targeted appropriately are significantly less nowadays, it may come with late complications, such as infertility. Replacing busulfan for antibody-based conditioning is an interesting development, that doesn't have these late effects. In the coming years, we expect to see more individualized, chemo-free, dosing regimens emerging in the field of HCT and GT. This way, outcome will be predictable and adjustable based on individual patients' needs and associated with very minimal short-term and long-term toxicity.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR
ADVANCED THERAPIES

SPOTLIGHT

EXPERT INSIGHT

Post-marketing safety and efficacy surveillance of cell and gene therapies in the EU: A critical review

Enrico Fritsche, Magdi Elsallab, Michaela Schaden, Spencer Phillips Hey, Mohamed Abou-El-Enein

The implementation of new regulatory tools, such as the PRIority MEdicine (PRIME) scheme, by regulatory authorities in Europe enabled faster patient access to innovative therapies. This early access tool goes along with a clear need for a thorough assessment of safety and efficacy upon marketing authorization. Due to the higher degree of uncertainty when evaluating novel therapies such as advanced therapy medicinal products (ATMPs), post-marketing surveillance studies for these products should be designed to make up the evidential shortfall and provide additional evidence to inform clinical practice. Here, we describe the status and regulatory requirements of post-marketing surveillance for ATMPs, which we found often resembling traditional, pre-market trials, focusing on biological mechanisms and efficacy in narrowly defined patient populations. We close by proposing the pragmatic trial concept as a potential solution to improve data quality and evidence generation in settings closer to real-world.

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FROM CLINICAL TRIALS TO POST-MARKET: THE CURIOUS CASE OF ATMPS

Evaluating a marketing-authorization application under the conventional centralized procedure of the European Medicine Agency (EMA) can be a lengthy process, taking up to 210 days (and that is excluding any additional time required for applicants to respond to EMA requests for additional information). In an effort to support drug development, the EMA has devised several early access routes for drug developers in the EU [1] where some of them can be utilized via the PRIority MEdicine (PRIME) scheme [2]. The PRIME scheme was launched in March 2016, specifically to support the more rapid translation of products targeting an unmet medical need by enhancing the early interaction and dialogue with regulators before submission of marketing authorization application (MAA), as well as accelerating the regulatory assessment procedure of MAA [3,4]. While such regulatory tools can help to more expeditiously satisfy unmet medical needs, this comes at the cost of having a less comprehensive data set, and therefore, greater uncertainty about the product's benefit-risk balance at the time of marketing authorization. However, to offset this initial lack of data, EMA obligates product developers to perform extensive post-marketing studies in order to generate more robust evidence supporting the overall safety and efficacy profile of these products. Similar policies are adopted by the US Food & Drug Administration (FDA) in cases when new drugs are approved on the basis of limited evidence [5,6]. Developers of advanced therapy medicinal products (ATMPs) have proactively integrated PRIME and other such regulatory tools into their product development strategies [7–9]. For example, by mid 2019, three ATMPs (Kymriah®, Yescarta®, and Zynteglo®) were approved under the PRIME scheme (although only Zynteglo benefited from the accelerated assessment; assessment of Kymriah and Yescarta were reverted to the standard timetable since major objections were raised during the regulatory evaluation and could not be resolved within the accelerated timetable).

However, the uncertainty about the benefit-risk profile of newly-approved ATMPs is not merely due to regulatory flexibility. There are several other features of clinical trials for ATMPs that can leave critical gaps in the evidence base concerning product safety and efficacy [10–15]. For example, since ATMPs often target rare diseases [16], the pre-market clinical trials are mostly small, single-arm trials that face an increased risk of bias and other translational challenges [17]. Surveys among ATMP-development companies in Europe have shown that for many rare diseases of interest, little is known about disease progression or the challenges associated with creation and interpretation of reliable endpoints for new indications [18]. Selection of endpoints is of particular importance since ATMP trials mainly rely on surrogate endpoints due to the lack of clinically meaningful ones for many indications, such as various cardiac cell therapy approaches [19]. However, relying on surrogate endpoints in pre-market trials only amplifies the uncertainty for how

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to appropriately use these products in the clinical setting [20].

To make up for this epistemic shortfall, post-marketing studies are therefore a critical tool for gathering the much-needed follow-up data, as well as allowing for additional evidence synthesis efforts to inform appropriate use of such products [21,22]. In order to better understand the characteristics of the post-marketing studies associated with ATMPs approval, we examined the regulatory landscape of post-marketing studies and performed a systematic review of the European Public Assessment Reports (EPAR) that is describing the evaluation of ATMPs authorized via the centralized procedure.

SEARCH STRATEGY AND SELECTION CRITERIA

Data on post-marketing clinical studies (planned and ongoing) of authorized ATMPs were extracted from the respective specific European public assessment report (EPAR) and the corresponding page in clinicaltrials.gov registry for each product. The EPAR search was based on the "find medicine" search function on the EMA website www.ema.europa.eu/en/medicines. The cut-off-date for data entry is November 1, 2019. Relevant data were extracted from the pharmacovigilance plan that, upon marketing authorization, each marketing authorization holder MAH has to provide within a detailed Risk management plan. Detailed information on this can be found within the EPAR, chapter "Risk Management Plan", sub-chapter "Pharmacovigilance Plan", which includes a table describing "type of study/ status", "categorization 1-3", "objectives", "safety concerns addressed", "date for submission of interim or final reports (planned or actual)" and optional "milestones" or separate information on "status". This sub-chapter represents 'additional pharmacovigilance activities', a regulatory term that encompasses all pharmacovigilance activities not considered as routine, and can include clinical studies or non-interventional post-authorization safety studies (more details provided in Box 1). These activities can be assigned at the time of marketing authorization to one of three categories that need to be followed when implementing post-marketing authorization studies on ATMPs. Category 1 is mandatory and comprises post-marketing studies that are imposed as conditions to the marketing authorization. These studies should provide key information to the benefit-risk profile of the product. Category 2 is also mandatory and entails specific obligations only in case of a conditional marketing authorization or a marketing authorization under exceptional circumstances. Finally, any other studies for investigating a specific safety concern or evaluating the effectiveness of risk minimization activities fall under category 3. Category 3 comprises activities which are conducted or financed by the MAH for investigating specific safety concerns, but 'do not include studies which are imposed or which are specific obligations' (i.e. excluding categories 1 or 2) [23].

Categorization of each ATMP to "Gene-", "Somatic cell-", or "Tissue-engineered" was based on information extracted from the EPAR of the respective authorized

BOX 1

The regulatory framework for post-marketing studies.

Traditionally, upon marketing authorization of a new ATMP, the identified risks and mitigation strategies have to be outlined in a separate risk management plan (RMP), as defined in the general 'Guideline on Good Pharmacovigilance Practices (GVP), Module V – Risk Management Systems (Rev2)' and an additional ATMP specific guidance on follow-up and risk management [57,58]. According to these guidelines, the marketing authorization holder should outline routine pharmacovigilance duties representing the primary/minimum set of activities required to fulfil the legal requirements contained in Directive 2001/83/EC [35]. However, due to the complex nature of ATMPs, the assessment of potential long-term safety concerns or lack of durable efficacy is of importance. These risks, such as germline transformation and vector transmission events or other genotoxic scenarios for genetically modified cell products, such as CAR-T cell products challenge the traditional pharmacovigilance systems. Therefore, a special focus is given on additional pharmacovigilance activities, as defined by the GVP guideline [57]. These activities should be performed in addition to the routine pharmacovigilance activities and Regulation (EC) No 726/2004 [59], and thus are expected to lead to a more informed benefit-risk balance [36]. If new safety data, indicating a substantial or potential risk, or new efficacy data under real-life conditions become available during conducted studies, follow-up measures have to be implemented accordingly and are legally binding.

Article 14(2) of Regulation (EC) No 1394/2007 provides a specific framework for RMPs related to ATMPs, which specifies how the MAH plans to further characterize the safety and efficacy concerns. Usually, the MAH addresses such additional activities by performing post-authorization safety studies (PASS) and/or post-authorization efficacy studies (PAES). Both types of studies have to be in line with Directive 2001/83/EC [35] and Regulation (EC) 726/2004 [59]. PASS usually aim to obtain further information on specifically identified safety concerns or to measure the effectiveness of the designed risk-management measures. PASS can either be an interventional or observational study, with the latter typically relying on "real-world data (RWD)" collected from registries, to aggregate and disseminate the long-term safety and efficacy data for ATMPs [25,26]. For instance, the cell therapy registries of the 'European Society for Blood and Marrow Transplantation (EBMT)' (EU) is one such registry that was modified to satisfy the requirement to collect more long-term outcome data on cellular therapies. The corresponding cellular therapy module of the EBMT registry recently got a qualification opinion by the EMA Committee for Medicinal Products for Human use (CHMP), describing the contexts in which EMA considers the use of registry data suitable [60]. PAES aims principally to further evaluate the efficacy of the approved products in order to gain more evidence on long-term product efficacy.

ATMP [24–33]. According to EMA classification, gene therapy products function by inserting 'recombinant' genes into the body, usually to treat a variety of diseases, including genetic disorders, cancer or long-term diseases. Somatic-cell therapy products contain cells or tissues that have been manipulated to change their biological characteristics or cells or tissues not intended to be used for the same essential functions in the body. Tissue-engineered products contain cells or tissues that have been modified so they can be used to repair, regenerate or replace human tissue [34,35].

The last published information on the post-marketing studies was collected by searching Clinicaltrials.gov database using the tradename, international non-proprietary name or, if available, clinicalTrials.gov identifiers (Data-cut-off: November 1, 2019).

CHARACTERISTICS OF POST-MARKETING STUDIES FOR ATMPS

The results of our data extraction for the 10 EMA-aproved ATMPs are presented in Table 1. The composition of the post-authorization studies of ATMPs was an equal split between interventional studies (50%) and observational studies (50%) (Figure 1). 35% of the interventional studies included were already ongoing at the time of marketing authorization (MA), and the applicant would be required to provide an update on the results of the studies, while 15% were newly designed studies. The newly planned interventional trials have generally adopted designs that resemble pre-market trials—eg., using single-arm designs, small sample sizes, or short-term follow-up periods with primary outcomes often focused on answering hypothesis from pre-marketing scenarios, focusing on a narrow study population rather than testing real-world scenarios in a broaden population (Table 1). Observational trials were either long term

TABLE 1

Overview of additional pharmacovigilance activities in post-marketing settings for all currently marketed ATMPs within Europe.

Class of ATMP	Name of ATMP (# of PMAS)	Category	Study design	Identifier	Pivotal (main study)	Sample size	Follow-up length	Study objectives to fulfil PMAS requirement	Status at time of MA
Somatic cell therapy medicinal products	Alofisel (2)	3	PASS	n.a.	X	n.a.	n.a.	Safety (Long-term)	Planned
		1	Phase III, randomized, double-blind, parallel-group, placebo-controlled, international, multi-center study (ADMIRE-CD-II)	NCT03279081	X	600	52 weeks	Safety (Long-term)	Ongoing
	Zalmoxis (4)	3	Interventional, Paediatric Investigation Plan (PIP) trial (TK009)	n.a.	X	n.a.	n.a.	Safety	Planned
		3	Interventional, PIP trial (TK010)	n.a.	X	n.a.	n.a.	Safety	Planned
		2	Phase III, randomized, interventional, open-label, clinical trial (TK008)	NCT00914628	X	170	12 months	Safety	Ongoing
		1	Non-Interventional PASS & PAES (TK011)	n.a.	X	n.a.	n.a.	Safety & effectiveness in real clinical practice Safety & efficacy (long-term)	Planned
Gene therapy medicinal products	Imlygic (5)	3	Registry study, observational, prospective cohort [Patient registry]	NCT02173171	X	340	3 years	Safety (long-term) Efficacy (long term)	Ongoing
		3	Post-marketing, prospective cohort study among patients treated in daily routine clinical practice	NCT02910557	X	920	5 years	Safety (long-term) Efficacy (long term)	Planned
		3	Phase II, Interventional, open-label, multicenter, single-arm trial (single group assignment)	NCT02014441	X	61	60 months	Biodistribution and Shedding of talimogene laherparepvec deoxyribonucleic acid (DNA)	Ongoing
		3	Phase I, multicenter, open-label, dose de-escalation study (single group assignment)	NCT02756845	X	18	24 months	Safety in pediatric subjects	Planned
		3	Randomized, controlled study	n.a.	X	n.a.	n.a.	Safety in pediatric subjects	Planned
	Kymriah (5)	1	Non-interventional, study (CCTL019B2401) with secondary use of data from two registries conducted by EBMT and CIBMTR	n.a.	X	n.a.	15 years	Safety (Long-term)	Planned
		1	Post-authorization efficacy study based on CCTL019B2401 observational registry study	n.a.	X	n.a.	n.a.	Efficacy and Safety (Real-world evidence data) in paediatric patients (< 3 years with B-ALL) treated in a commercial setting	Planned
		1	Prospective, observational PAES study in DLBCL(C2201)	n.a.		n.a.	n.a.	Efficacy follow-up in r/r diffuse large B-cell lymphoma (DLBCL), patients evaluated in study C2201	Ongoing
		1	Study CCTL019H2301, a randomized open-label parallel-group multicenter Phase III trial	NCT03570892	X	318	5 years	Efficacy	Planned
		3	Study CCTL019A2205B, Long-term follow-up, observational, registry, non-randomized, open-label, single group assignment	NCT02445222	X	1250	15 years	Safety (Long-term)	Ongoing
	Luxterna (2)	1	Post-authorization, multicenter, multinational, longitudinal, single-group, prospective, observational, safety registry study (SPKRPE-EUPASS)	n.a.	X	n.a.	n.a.	Safety (Long-term)	Planned
		1	Observational, multi-site, non-randomized, prospective cohort, long-term safety and efficacy follow-up study (LTFU-01)	NCT03602820	X	41	15 years	Safety (Long-term), Efficacy (Long-term)	Ongoing
	Strimvelis (4)	1	Long-Term, Prospective, Non-Interventional Follow-up of Safety and Efficacy, patient registry study (200195)	NCT03478670	X	50	15 years	Safety (Long-term)	Ongoing
		3	Long-term follow up of patients from study AD1115611	NCT00598481		18	4 to 8 years	Safety (Long-term)	Ongoing
		3	Surveys to HCPs/PIDs and parents/carers of pediatric patients	n.a.	X	n.a.	n.a.	Effectiveness of additional risk minimization measures (e.g. educational materials)	Planned
		3	Post-marketing approval methodology study	n.a.	X	n.a.	n.a.	RIS analysis to predict malignancy due to insertional oncogenesis	Planned
	Yescarta (9)	1	Non-Interventional Registry Study (PASS)	n.a.	X	n.a.	n.a.	Safety	Planned
		3	Prescriber survey	n.a.	X	n.a.	n.a.	Safety	Planned
		3	Interventional, Phase I/II, multicenter, open-label study (single group assignment) (ZUMA-1)	NCT02348216		290	12 to 24 months	Safety, Efficacy	Ongoing
		3	Interventional, Phase II, multicenter, open-label study (single group assignment) (ZUMA-2)	NCT02601313	X	105	15 years	Safety (Long-term), New indication: r/r Mantle Cell Lymphoma (MCL)	Ongoing
		3	Interventional, Phase I/II, multicenter, open-label study (single group assignment) (ZUMA-3)	NCT02614066	X	125	24 months	Safety (r/r Acute Lymphoblastic Leukemia (ALL))	Ongoing

The identified studies were colour coded to highlight their type as follows: Interventional studies planned at time of marketing authorization, **Interventional studies ongoing at time of marketing authorization**, Observational studies planned at time of marketing authorization, **Observational studies ongoing at time of marketing authorization.**

ADMIRE-CD, Adipose-derived mesenchymal stem cells for induction of remission in perianal fistulizing Crohn's disease; ALL, Acute lymphoblastic leukemia; ATMP, Advanced therapy medicinal product; CIBMTR, Center for International Blood & Marrow Transplant Research; CNS, Central nervous system; DLBCL, Diffuse large B-cell lymphoma; DNA, Deoxyribonucleic acid; EBMT, European Society for Blood and Marrow Transplantation; HCP, Healthcare provider, LTFU, Long-term follow-up; MA, Marketing authorization; MCL, mantle cell lymphoma; MF, Microfracture; n.a., not applicable; NHL, Non-Hodgkin lymphoma; PAES, Post-authorization efficacy study; PASS, Post-authorization safety study; PIP, Paediatric Investigation Plan; PMAS, Post-marketing authorization studies; RIS, Retroviral Insertion Site; r/r, relapsed/refractory; TDT, Transfusion-dependent β-thalassaemia.

TABLE 1 (CONT.)

Overview of additional pharmacovigilance activities in post-marketing settings for all currently marketed ATMPs within Europe.

Class of ATMP	Name of ATMP (# of PMAS)	Category	Study design	Identifier	Pivotal (main study)	Sample size	Follow-up length	Study objectives to fulfil PMAS requirement	Status at time of MA
Gene therapy medicinal products (CONT.)	Yescarta (9) (CONT.)	3	Interventional, Phase I/II, multicenter, open-label study (single group assignment) (ZUMA-4)	NCT02625480	X	100	24 months	Safety (pediatric r/r ALL patients)	Ongoing
		3	Interventional, Phase II, multicenter, open-label study (single group assignment) (ZUMA-5)	NCT03105336	X	160	15 years	Safety (Long-term) (r/r indolent NHL)	Ongoing
		3	Interventional, Phase I/II, multicenter, open-label, supportive study (single group assignment) (ZUMA-6)	NCT02926833	X	37	5 years	Safety in combination with Atezolizumab (Long-term) (refractory DLBCL)	Ongoing
		3	Interventional, Phase III, randomized, open-label, multicenter study (parallel assignment) (ZUMA-7)	NCT03391466	X	359	5 years	Safety (Long-term)	Ongoing
	Zynteglo (4)	1	Long-term observational registry study (including product registry REG-501)	n.a.	X	n.a.	n.a.	Safety (Long-term) Efficacy (Long-term)	Planned
		2	Single-arm interventional, phase III, open-label study (HGB-207)	NCT02906202		23	24 months	Safety, Efficacy (transfusion-dependent ß-Thalassemia (TDT) patients without ß0/ß0 genotype)	Ongoing
		2	Single-arm interventional, phase III, open-label study (HGB-212)	NCT03207009		15	24 months	Safety, Efficacy (TDT patients with ß0/ß0 genotype)	Ongoing
		2	Prospective, observational case-only, long-term follow-up study (LTF-303)	NCT02633943		94	15 years	Safety (Long-term), Efficacy (Long-term)	Ongoing
Tissue-engineered products (TEP)	Holoclar (3)	2	Multinational, multicentre, prospective, open-label, uncontrolled study (HLSTM03)	n.a.	X	n.a.	n.a.	Safety (Long-term), Efficacy (Long-term)	Planned
		3	Long-term safety and efficacy follow-up study connected to HLSTM03 (HLSTM03FU)	n.a.	X	n.a.	n.a.	Safety (Long-term), Efficacy (Long-term) Success after keratoplasty	Planned
		3	Post-authorization observational, patient registry study during routine clinical practice	n.a.	X	n.a.	5 years	Safety (Long-term) under routine clinical conditions	Planned
	Spherox (2)	n.a.	Prospective, randomized, open-label, multicentre Phase III clinical trial (cod 16HS13)	NCT01222559		102	60 months	Safety (Long-term), Efficacy (Long-term) (Compare to active comparator 'microfracture' (MF))	Ongoing
		n.a.	Prospective, randomized, open-label, multicentre Phase II clinical trial (cod 16HS14)	NCT01225575		75	60 months	Safety (Long-term), Efficacy (Long-term) (Compare three different doses)	Ongoing

The identified studies were colour coded to highlight their type as follows: Interventional studies planned at time of marketing authorization, **Interventional studies ongoing at time of marketing authorization,** Observational studies planned at time of marketing authorization, **Observational studies ongoing at time of marketing authorization.**

ADMIRE-CD, Adipose-derived mesenchymal stem cells for induction of remission in perianal fistulizing Crohn's disease; ALL, Acute lymphoblastic leukemia; ATMP, Advanced therapy medicinal product; CIBMTR, Center for International Blood & Marrow Transplant Research; CNS, Central nervous system; DLBCL, Diffuse large B-cell lymphoma; DNA, Deoxyribonucleic acid; EBMT, European Society for Blood and Marrow Transplantation; HCP, Healthcare provider, LTFU, Long-term follow-up; MA, Marketing authorization; MCL, mantle cell lymphoma; MF, Microfracture; n.a., not applicable; NHL, Non-Hodgkin lymphoma; PAES, Post-authorization efficacy study; PASS, Post-authorization safety study; PIP, Paediatric Investigation Plan; PMAS, Post-marketing authorization studies; RIS, Retroviral Insertion Site; r/r, relapsed/refractory; TDT, Transfusion-dependent β-thalassaemia.

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follow up of the patients treated in premarketing studies or mostly a registry study to collect data on particular safety and efficacy parameters in real-world settings.

The number of total post-marketing studies ranged from two to nine across the 10 ATMPs (Figure 2). The sample size of the trials ranged from 15 to 1250 patients (Table 2), with most of the trials intending to enrol fewer than 200 patients (Figure 3). Duration of follow-up in trials ranged from 12 to 180 months (Table 2), which is mostly dependent on the use of viral vectors in the treatment since these products require longer follow-up for safety-related

FIGURE 1

Percentage of post-marketing authorization study types at MA for all marketed ATMP.

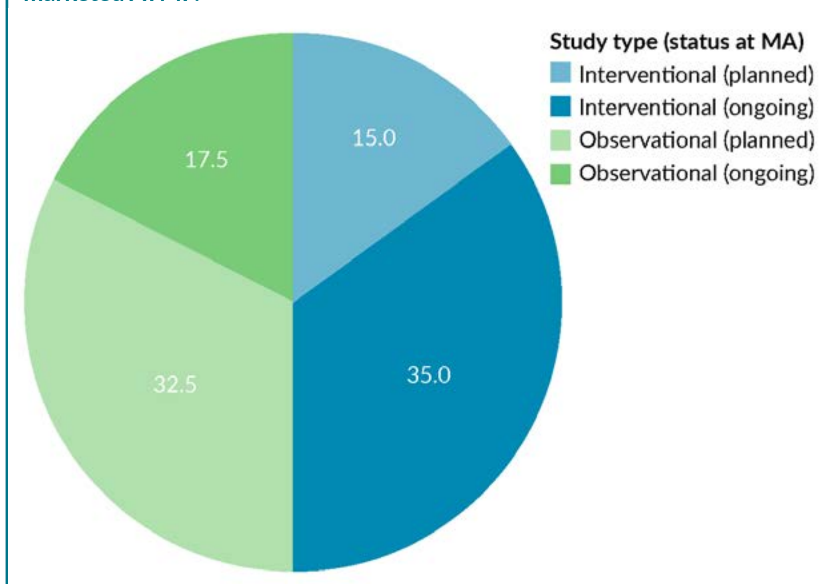
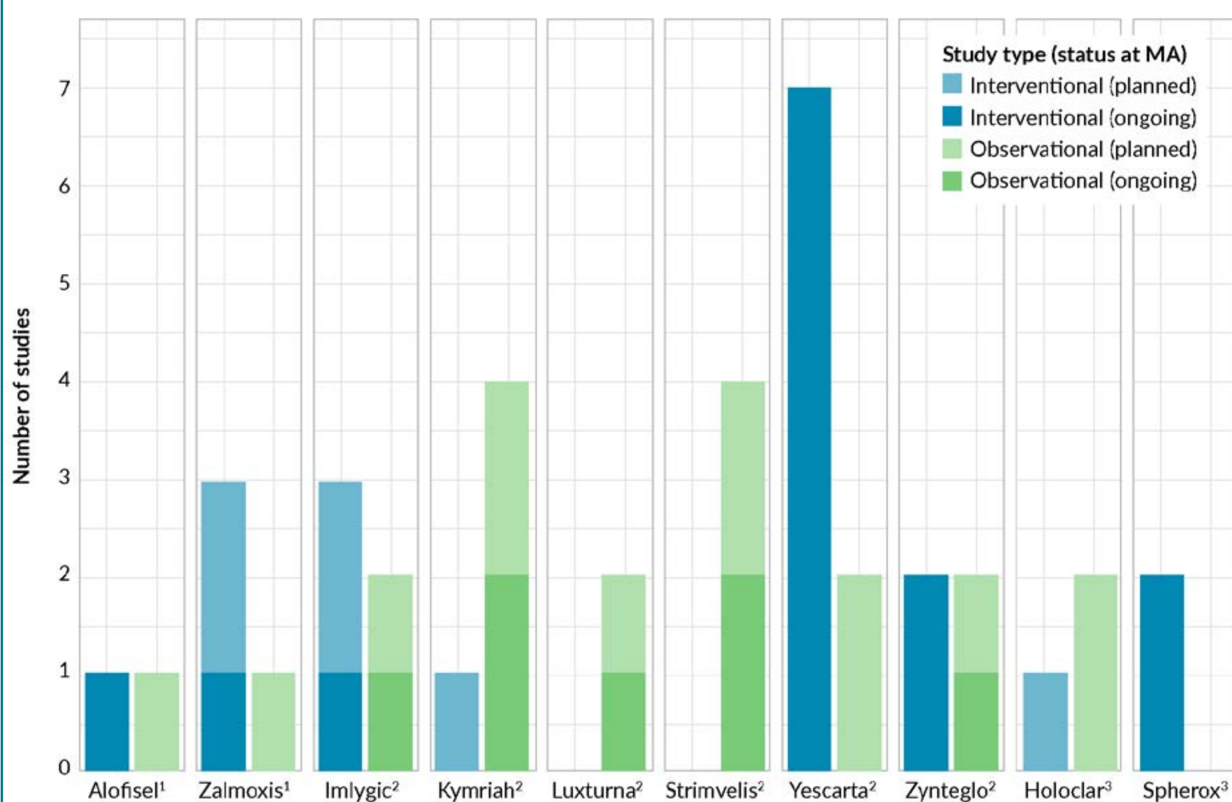


FIGURE 2

Number and types of post-marketing authorization studies submitted by each applicant.



ATMPs were categorized according to the regulatory class as follows:
(1) Cell therapy medicinal product (CTMP)
(2) Gene therapy medicinal product (GTMP),
(3) Tissue-engineered product (TEP).

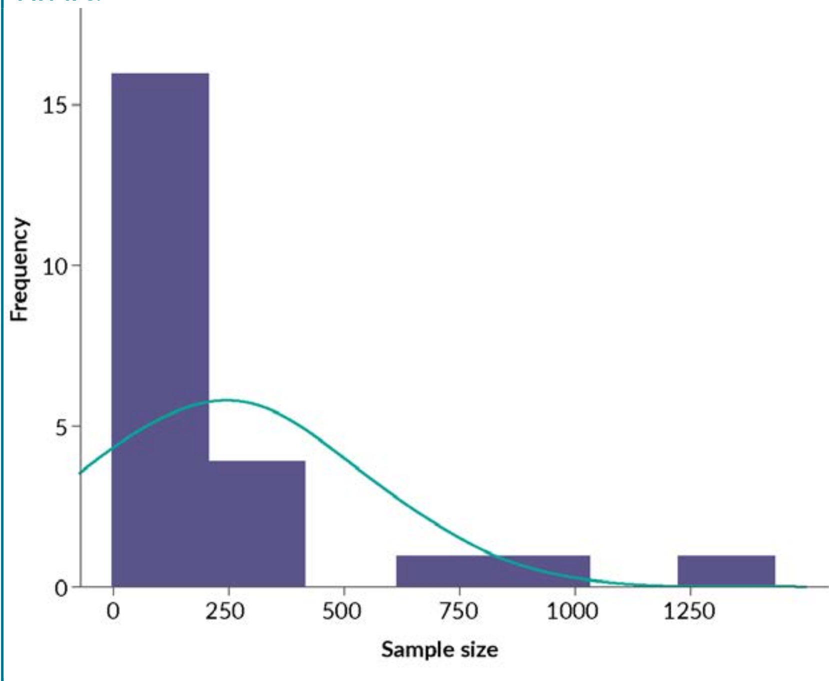
TABLE 2.

Summary statistics of post-marketing authorization design parameters among ATMPs.

		Sample size	Follow-up (months)
N	Valid	23	25
	Missing	2	0
Mean		229,17	80,44
Median		102,00	60,00
Std. Deviation		310,228	65,775
Minimum		15	12
Maximum		1250	180

FIGURE 3

Frequencies of sample sizes specified in the post-marketing studies of ATMPs.



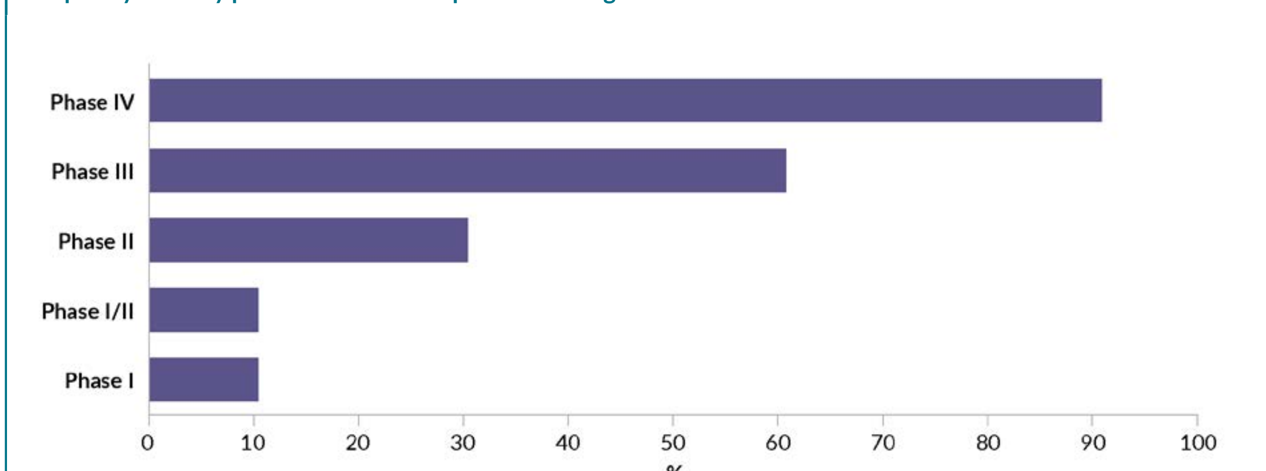
parameters. Half of applicants (50%) included Phase III interventional trial designs that traditionally focus on product efficacy as a primary endpoint and usually form the basis of the regulatory submissions and authorization (Figure 4). Most of these trials (86%) were initiated before the MA but only a few of them represent pivotal trials upon which the MA was acquired and the rest continued to perform these trials as part of the post-marketing surveillance phase which would have traditionally been included in a MA submission package (Table 1).

CURRENT POST-MARKETING TRIALS FOR ATMPS RATHER ATTAIN PRE-MARKETING DESIGNS

Our analysis suggests that a high degree of variability between trial designs of ATMPs in post-marketing settings cthat can be explained by the wide range in evidence generated from clinical studies at the time of MAA, the rarity of the indication and the specific characteristics of the product itself (whether

FIGURE 4

Frequency of study phases submitted as post-marketing authorization studies.



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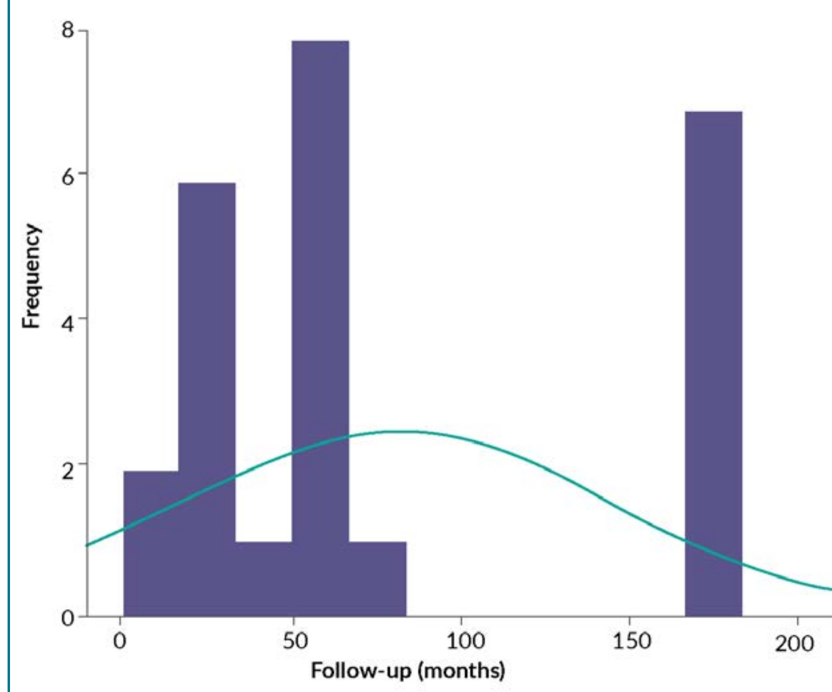
it is genetically modified or not). This inherited variability requires developers to devise a post-marketing strategy based on a case-by-case scenario. Moreover, many of the post-marketing trials, intended to address the critical benefit-risk knowledge gaps for ATMPs, are phase III interventional trials that may not be well suited to inform routine clinical use of the products. The sample sizes of post-marketing trials are relatively small for studies that should aim to reflect real-life situations. Further analysis of the data also showed high variability in the follow-up periods specified for each study (Figure 5). These observations add the challenge of identifying a clear cut-off between pre- and post-marketing studies and the exact added value of PMAS.

The dominant trial paradigm for decades has been explanatory in its orientation—which is to say that trials have been designed to test experimental interventions under "idealized" or "laboratory-like" conditions that are optimized to detect a treatment effect. Explanatory trial designs thus generally involve strict intervention protocols, many patient inclusion/exclusion criteria, and high-resourced settings in an effort to control for systemic errors (e.g. confounding, bias) and deliver statistically credible results of high internal validity [36]. In the pre-market setting, this approach to trial design makes good sense, since the primary question from a regulatory perspective may be of the form: How will this new intervention work for this particular patient in a controlled setting? By contrast, in the post-market setting, the primary question is a different form: "Can this new intervention be beneficial once available to the wider population?" Addressing this question requires a more pragmatic orientation to trial design, which often involves more flexible protocols, broader and more heterogeneous patient populations, and a mix of different healthcare settings. For instance, early evidence of the real-world efficacy performance of Yescarta, a CAR T cell-based product used in treatment of non-Hodgkin lymphoma (NHL), showed that the efficacy signals generated once the product was used in clinical practice, applied to more heterogeneous population are slightly inferior to that generated from the clinical trial [37].

This raises the question of whether it is more sensible to continue reproducing data in post-marketing settings within the framework of phase III-type trials or rather introduce more flexible designs that would account for real-world heterogeneity.

FIGURE 5

Frequencies of follow-up periods specified in the post-marketing studies of ATMPs.



PRAGMATIC CONCEPTS, A PROMISING OPTION TO ENHANCE POST-MARKETING TRIALS

Pragmatic trials are meant to inform a clinical or policy decision by evaluating the effectiveness of interventions in real-world clinical practice [38]. We believe that the distinction between explanatory and pragmatic trial concepts may be valuable here when designing post-marketing trials that are needed to ensure safe and reliable use of ATMPs and fulfil the requirements of multiple stakeholders (regulators, HTA bodies, payers etc.) [39].

For ATMPs, the major drawbacks of an explanatory orientation are the small sample size due to low incidence levels, a short time frame of observation, limited or complete exclusion of distinct patient population such as vulnerable populations (e.g., children, elderly and pregnant women) and patients with comorbidities (e.g., neurological or hematological disorder, autoimmune disease or infections) [40]. More heterogenic outcome data would be of particular value for these products, due to commonly underlying heterogenic baseline parameters immanent to the types of rare diseases that are frequently of interest. Thus, it seems that more pragmatic trials would be suitable for ATMP post-marketing safety and efficacy surveillance. Although the data from pragmatic trials is noisier, it can nevertheless provide a more representative picture of whether an intervention actually has utility in clinical practice (or how its utility may vary from one set of conditions to the next).

However, when characterizing explanatory and pragmatic trial concepts, it is crucial to observe that the explanatory/pragmatic distinction is not a dichotomy, but a multi-dimensional continuum. In recognition of this point, Thorpe *et al.* implemented the PRagmatic-Explanatory Continuum Indicator Summary (PRECIS) tool, which breaks down a trials pragmatism (or lack thereof) along 10 different dimensions as described in their published report [41].

As a result of subsequent extensive discussion on the concept of pragmatism within clinical research methodology [40,42–50], the requirements for characterizing a study as pragmatic trial were optimized and validated, leading to the implementation of PRECIS-2 [51]. This improved tool represents a nine-spoked 'wheel' with nine domains based on trial design decisions. The features of PRECIS-2 were summarized in Box 2.

BOX 2

The Pragmatic-Explanatory Continuum Indicator Summary 2 (PRECIS-2) wheel (adapted from Loudon *et al.*, 2015 [51]).

1. **"Eligibility" domain** should describe who is selected to participate in the trial.
2. **"Recruitment" domain** describes how are participants recruited into the trial.
3. **"Setting" domain** includes information on where is the trial being done.
4. **"Organisation" domain** provides information on what kind of expertise and resources are needed to deliver the intervention.
5. **"Flexibility: delivery" domain** describes how the intervention should be delivered.
6. **"Flexibility: adherence" domain** comprises information on what measures are in place to make sure participants adhere to the intervention.
7. **"Follow up" domain** describes how closely are participants followed-up.
8. **"Primary outcome" domain** provides information on how relevant findings are to participants
9. **"Primary analysis" domain** should summarize to what extent all data are included.

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AN EXEMPLARY APPLICATION OF PRAGMATIC TRIAL DESIGN TO CAR-T CELL THERAPEUTICS

To concretely illustrate how we believe pragmatic, post-marketing trials for ATMPs should be designed, we applied a PRECIS-2 analysis to hypothetical trials for the two marketed CAR-T cell therapies, tisagenlecleucel (Kymriah®, Novartis) and axicabtagene ciloleucel (Yescarta®, Kite Pharma/Gilead). In what follows, we discuss particular PRECIS-2's dimensions that are relevant to our case study and how we believe trials of Kymriah and Yescarta should be oriented along the pragmatic spectrum.

Eligibility criteria

Current post-marketing studies for Yescarta and Kymriah relied on restricted enrollment according to the authorized indication with deliberate consideration of contraindications, special warnings, and precautions for use (see Annex I, Summary of product characteristics) [52,53]. An exception to this was given in case of enrolling special patient populations not covered at marketing authorization, thereby addressing missing information according to the RMP (e.g. use in HBV/HCV/HIV infection, use in patients with active Central Nervous System (CNS) involvement in malignancy). However, to be more pragmatic and better inform clinical use, we believe that there should be greater flexibility in the eligibility criteria, for example, allow for patients with different schemes of pre-conditioning lymphodepleting therapies. This flexibility will enable further exploration of the effects of different treatment schemes on the product outcome, and thereby filling some of the critical information gaps for distinct patient populations, e.g. co-morbidities, variable pre-treatment or age groups. The EBMT registry could be useful here as well since it enables entering standardized data that can be analyzed in larger post-marketing studies.

Organization

To deliver clear information on what kind of expertise and resources are needed to deliver the intervention of interest, current approaches to post-marketing trials tend to focus on interventions being only performed by physicians/hospitals that are specially trained within the control distribution program as a risk minimization measure. Given the risks associated with Kymriah and Yescarta, we believe that this is the right approach and that a more pragmatic design along this dimension is not necessary.

Flexibility of delivery

Relating to mechanisms on how to deliver the intervention, current post-marketing concepts for Kymriah and Yescarta CAR-T cell products focus on administration only within the authorized dosing regimen. However, clinicians may often have to adjust dosing in practice, for example, for patients with low baseline T-cell concentrations in leukapheresis starting materials. A more pragmatic approach would thus include administration of products that do not meet the commercial specifications. However, an

important pre-requisite to allow for this kind of approach would be that no overwhelming safety concerns have been identified during manufacture and release of such out-of-specification (OSS) product. This would enable a deeper investigation of the dose-response relationship for OSS concentration levels of the CAR-T cell product. For instance, the authorized dosage of Kymriah is 0.2-5x10^6 CD19+ CAR T-cells (body weight-based), with a maximum dose of 2.5x10^8 CAR T-cells (non-body weight-based). However, even lower dose ranges (e.g. ≤0.03 x 10^6) in acute lymphoblastic lymphoma trials showed a clinical response [54].

Flexibility of adherence

A pragmatic approach here would ideally deviate as little as possible from standard practice by avoiding highly stringent time frames for study visits, thus increasing flexibility and patient adherence. Less stringent follow-up visits may be also motivating for practitioners, in reducing the associated monitoring and workload without jeopardizing patients safety.

Follow up

The procedure for follow up under current post-marketing settings relies on an evaluation period of up to 15 years for safety and efficacy surveillance after CAR-T cell administration. When applying pragmatic trial concepts in the post-marketing settings, post-market studies need to interface with the patient registries and rely on them, which in turn will be the most pragmatic 'information engine' in the long-run. This has the potential to enhance patient compliance and commitment for a longer follow up period and promote patient consent to register data in the EBMT registry and use it, for instance, as a source of external control data for comparative purposes.

Primary outcome

The current post-marketing trials for Kymriah and Yescarta aim at further characterization of safety profiles, specifically related to Cytokine Release Storm (CRS), neurotoxicity, infections, prolonged cytopenias, growth and development, reproductive status and pregnancy outcomes. Some trials aim to characterize further the efficacy profile related to Overall Response Rate (ORR), CD19 CAR T-cell level, incidence/exacerbation of pre-existing comorbidities, relapse/progress disease, incidence death and monitoring of replication-competent lentivirus. A pragmatic approach along this dimension could apply a more concise set of outcomes, particularly the ones that interfere with patients' daily productivity and quality of life while keeping additional tests or visits to a minimum. In order for the pragmatic approach to provide added value outside of the controlled environment of an explanatory trial, it should also incorporate relevant patient decision-making criteria to provide meaningful evidence and build upon the evidence generated at the time of MAA.

Primary analysis

There was no access to detailed statistical analysis plans to evaluate

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primary analysis mechanisms under the current conventional post-marketing setting for Kymriah and Yescarta. For a pragmatic trial approach here, including a heterogeneous patient population and planning for sub-group analyses would allow for detecting clinically-relevant safety and efficacy signals. Indeed, given the limited data that is often available for ATMPs, we would recommend a maximally pragmatic approach to the primary analysis of post-marketing trials to try and include as much patient-relevant data as possible.

CONCLUDING REMARKS AND FUTURE CONSIDERATIONS

While the new regulatory tools developed by EMA to facilitate rapid marketing authorization in cases of major public health interests are warranted, these tools must be accompanied by sound post-authorization strategies to generate long term evidence for safety and efficacy.

The current regulatory landscape for conducting post-authorization studies is very complex and demands an enormous effort from the MAH to navigate. As we have shown, there is a wide variety in post-market study designs for ATMPs, both for studies that are required by the regulatory authorities and for those studies conducted voluntarily by the MAH to investigate a specific safety concern or to evaluate the efficacy/effectiveness of risk minimization activities (classified in category 1, 2 and 3). However, within that variety, we observed that many post-marketing surveillance trials for ATMPs adopt explanatory trial design features and are focusing on answering hypotheses that a more suitable to pre-market trials. Thus, we believe that there is room for improvement in terms of designing (or mandating) post-market trials of ATMPs that will better meet the informational needs of patients, clinicians, and payers.

We have suggested that applying the tools of pragmatic trial design (as made explicit by the PRECIS-2 framework) may help to fill this gap. In particular, RWD generated from registry-based pragmatic trials would have great potential to generalize findings and better inform the use of ATMPs across diverse patient populations. However, there are certainly challenges regarding data quality, as patient populations will become more divergent, thus increasing the risk for confounding and a higher degree of diffuse data generated. On the other hand, the aggregation of clinical data collected from RWD can increase the robustness of meta-analyses derived from post-marketing studies. In a recent report, regulators from the Swedish Medical Products Agency called for more attention to methodological basics of post-marketing studies that can help generate reliable results and affirmed the regulatory value of the pragmatic trial concept regardless of labelling the studies as 'pragmatic' or 'real-world evidence' [55]. Moreover, the field of ATMP may learn from medical device evaluations, where regulatory agencies already implemented guidance on how to apply real-world data for regulatory decision-making [56].

While all aspects of the approach of a pragmatic trial do not need to be implemented at once, working on improving the current post-marketing study methodology and supplementing it with new ideas can help

to enhance the current practice (Box 3). We believe that such an approach would not only be beneficial for MAHs but also for regulators and health insurance providers, to obtain real-world data from clinical routine faster after marketing authorization for ATMPs.

BOX 3

Considerations for using pragmatic trial concepts for post-authorization safety and efficacy studies (both observatonal and interventional).

1. Closing the evidence gap between Early Access Program (EAP) mediated marketing authorization for innovative medicines and the lack of data on their long-term safety and efficacy at the time of marketing authorization
2. Gaining data on a more heterogeneous study population and none or only partially addressed risks at an earlier stage after marketing authorization in a setting more closer to real-time and real-life scenarios
3. Implementation of patient broad consent to share data among registry studies
4. Reducing enormous timely and regulatory effort by submitting more broaden study protocols for pragmatic trial instead of numerous single protocols for individual conventional explanatory studies
5. Pooling of study objectives in combination with implementing patient registry to enter standardized data sets and share them for statistical analysis in sub-groups
6. Addressing pharmacovigilance requirements of GVP module V, referred to in the post-authorization development plan as part of the risk management plan
7. Conferring economic benefits for MAH, regulators, and health insurance providers by accelarating the process for obtaining real-world data from clinical routine upon marketing access
8. Allowing for taking into account more/all variabilities on patient and product level (e.g. patient baseline status, product design) to potentially enabling optimization of patient access

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR ADVANCED THERAPIES

SPOTLIGHT

EXPERT INSIGHT

CRISPR surgery for inherited retinal diseases: landmarks in the 21st century

Alexander H Chai & Stephen H Tsang

Gene therapy was first conceptualized in 1972 as clarification on viral DNA-altering mechanisms was done. Since then, the field of gene therapy has transformed from a biological fantasy into a valid clinical treatment in humans, in part due to significant innovations in the field of molecular genetics. The development of gene therapy technology and the ensuing research has laid a strong foundation for the advancement of gene therapy, which has the potential to correct dominantly inherited disorders that were previously incurable. In November 2018, a drug named Luxturna became the first *in vivo* CRISPR/Cas9 genome surgery treatment to be FDA-approved for use in clinical trials, which are set to take place in patients with Leber congenital amaurosis 10 in the fall of 2019 [1]. However, there remain a number of scientific and practical barriers to resolve before genomic medicine can become a widespread treatment.

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PROGRESS IN CRISPR THERAPEUTICS

Gene therapy was first conceptualized in 1972 as research was conducted on the DNA-altering mechanisms of viruses [2]. Since then, genomic medicine has slowly transformed into valid treatment for a limited number of genetic diseases, particularly recessive loss-of-function disorders [3]. Most FDA-approved gene therapy trials

have been targeted at diseases with an autosomal-recessive mode of inheritance, where researchers have found that supplementation with the wild-type version of the mutant gene may restore healthy function in cells. This method is called gene augmentation. Unfortunately, this supplementation method only works for recessive disorders – autosomal-dominant (gain-of-function) disorders do not respond the same way. A number of treatments are being researched for dominant disorders, including RNA interference therapeutics, but currently the most developed hope for a cure is genome surgery to repair or remove DNA (Table 1).

Fortunately, recent advancements in molecular genetics, particularly the progress of CRISPR (clusters of regularly-interspaced short palindromic repeats)/Cas9 technology, have provided this hope. CRISPR/Cas9 technology became popular in 2013 when it was shown to successfully edit the DNA of human cells; since then, researchers have been focusing on the development and refinement of this technology for clinical purposes [4]. CRISPR's greatest advantage over other gene-editing technologies is its low cost and high efficiency when compared to other techniques such as transcription activator-like effector nucleases (TALEN) or zinc-finger nucleases (ZFN) [5]. This is mainly due to the fact that CRISPR's cutting mechanism is guided by a strand of RNA, which is much simpler to engineer than the complex proteins which ZFN and TALEN technologies rely on [6]. CRISPR is also the only one of these technologies capable of targeting more than one genetic location via multiplexed genome surgery [7]. This is done by packaging multiple specific guide RNAs into the viral delivery vectors, therefore guiding the cutting enzyme to multiple locations in the genome [8].

Ophthalmology in particular has become the leading field for the development of genomic medicine; the eyes are very favorable targets for these treatments and testing. The eye's duplicity enables researchers to test the effects of treatments on one eye while having a dynamic control to compare the natural progression of disease with, and they don't require invasive procedures for treatment or observation. The eye also has a special relationship with the immune system. The eye exhibits a reduction in antigen-presenting cells and immunomodulatory factors in the vitreous humor when compared with other cells, which allows it to better tolerate the administration of gene surgery vectors [9]. Immunosuppressive cytokines and surface molecules displayed on ocular parenchymal cells, which interact with regulatory T cells to dampen inflammatory responses, also contribute to the eye's relatively immune-privileged state and ability to tolerate gene therapy [10,11].

In December of 2017, the first *in vivo* gene therapy was approved by the FDA for treatment of patients with Leber congenital amaurosis 2 [12]. The drug, Luxturna (voretigene neparvovec-rzyl), is composed of an adeno-associated virus containing human RPE65 cDNA and is delivered subretinally. It was first sold commercially in March of 2018, and was a groundbreaking step towards the widespread use of gene therapy as a treatment in humans.

More recently, in November 2018, a groundbreaking *in vivo* CRISPR/Cas9 treatment for Leber

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TABLE 1

Ongoing current ocular gene augmentation/surgery trials.

Disease	Treatment	Phase	End date	Sponsor
Achromatopsia	Subretinal administration of rAAV2tYF-PR1.7-hCNGB3	1/2	2022	Applied Genetics Technologies Corp.
Atrophic age-related macular degeneration	RPE transplantation	1/2	2020	Chinese Academy of Sciences
Choroideremia	Subretinal administration of AAV2-REP1	2	2021	Bryon Lam
	Subretinal administration of AAV2/REP1	2	2021	University of Oxford
	Subretinal administration of AAV2-hCHM	2	2019	Spark Therapeutics
	Subretinal administration of AAV2-REP1	3	2020	Nightstar Therapeutics
Leber congenital amaurosis 2 (LCA2)	Subretinal administration of AAV2-hRPE65v2	3	2029	Spark Therapeutics
		1	2024	Spark Therapeutics
		1/2	2026	Spark Therapeutics
	Subretinal administration of AAV2/5 OPTIRPE65	1/2	2023	MeiraGTx UK II Ltd.
	Subretinal administration of rAAV2-CBSB-hRPE65	1	2026	University of Pennsylvania
Leber congenital amaurosis 10 (LCA10)	Subretinal administration of AGN-151587	1/2	2024	Editas/Allergan
Leber hereditary optic neuropathy (LHON)	Intravitreal administration of GS010 (rAAV2/2-ND4) versus sham intravitreal administration	3	2019	GenSight Biologics
			2019	GenSight Biologics
			2021	GenSight biologics
	Intravitreal administration of scAAV2-P1ND4v2	1	2019	John Guy, University of Miami
Neovascular age-related macular degeneration (AMD)	Subretinal administration of RGX-314	1	2020	Regenxbio Inc.
	Intravitreal administration of ADVM-022	1	2022	Adverum Technologies
	Subretinal RetinoStat		2027	Oxford Miomedica
Stargardt disease	Subretinal administration of SAR422459	1/2	2019	Sanofi
			2034	Sanofi
Usher syndrome 1B	Subretinal administration of UshStat (EIAV-CMV-MYO7A)	1/2	2021	Sanofi
		1/2	2036	Sanofi
X-linked retinitis pigmentosa (XLRP)	Subretinal administration of rAAV2tYF-GRK1-RPGR	1/2	2024	Applied Genetic Technologies Corp
	Subretinal administration of AAV2/50hRKp.RPGR	1/2	2020	MeiraGTx UK II Ltd.
	Subretinal administration of AAV-RPGR	1/2	2019	Nightstar Therapeutics
X-linked retinoschisis	Intravitreal AAV8-scRS/IRBPhRS	1/2	2021	National Eye Institute
	Intravitreal rAAV2tYF-CB-hRS1	1/2	2022	Applied Genetic Technologies Corp.

Information in table sourced from clinicaltrials.gov and DiCarlo *et al.* [18].

congenital amaurosis 10 was approved by the FDA for Phase 1 trial, the first approved *in vivo* use of CRISPR technology in humans [13]. Patients in the trial will be treated by subretinal injection of AGN-151587 in one eye, which should theoretically cut out the malfunctioning gain-of-function mutation and repair the malfunctioning CEP290 gene. This gene codes for a protein vital in the development of ciliogenesis, radial microtubule organization, and centriolar satellite clustering [14]. This gene, via knockout studies, has been shown to be vital for the development of healthy photoreceptor cells. This trial is a landmark development in the field of genetic medicine, as the safety and efficacy of CRISPR technologies directly injected into humans can finally be evaluated. Pending the results of this early investigation, this study could provide framework for further development of novel CRISPR-based genome surgery treatments of inherited retinal diseases and propel further research on the safety and efficacy of CRISPR-based treatments in other organ systems.

TRANSLATION INSIGHT

There are a number of questions to be answered surrounding the practicality of precision genome surgery. First-generation CRISPR/Cas9-based genome surgery's greatest strength, its extreme mutation specificity, is also its greatest weakness. Clinical gene therapy trials for autosomal dominant retinitis pigmentosa (adRP) that focus on repair of a single gene, even if successful, would only be applicable to patients carrying that specific gene mutation. Each treatment must cut and replace only one gene out of the estimated 30,000 genes in the human genome [15], or the patient could suffer from extreme side effects. For a disease like adRP, a blinding disease caused by any one of the 150 mutations in the RHO gene discovered so far [16], it would take millions of dollars and several decades to develop the 150 gene-specific treatments to cure patients with just this one disease. Additionally, each gene-specific treatment must be individually approved by the FDA after years of trials proving the safety and efficacy of the treatment. With such a broad spectrum of pathogenic mutations and a relatively small number of patients with each mutation, it would be financially unsustainable to develop such a wide variety of highly specific genome surgery treatments. Due to these factors, there is currently no effective therapeutic option for patients with adRP or any other patient with gain-of-function photoreceptor degenerations.

Additionally, even with significant advancements, today's CRISPR/Cas9 technology also contains an inherent risk of off-targeting that cannot be fully addressed by today's technology. Off-target gene ablation can have significant side effects, possibly even death. These effects are difficult to predict and mitigate; although technologies can determine most of the off-target sequences in a genome, current methods are not guaranteed to comprehensively identify all of these sequences [17]. In addition to the costs of advanced bioinformatics and next-generation sequencing technologies, the results are confounded by the high degree of variation of genetic material within each individual [17].

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These risks must be evaluated on an individual basis with patients considered for CRISPR-based genome surgery.

There have been some efforts at correcting gain-of-function diseases using mutation nonspecific methods that can fix faulty DNA at specific sites, but these methods cannot completely replace traditional CRISPR methods. One method, referred to as CRISPR2.0, delivers a second viral vector with wild type cDNA that is modified to introduce mismatches through silent mutations and makes it resistant to gRNA targeting. This design ensures that gene ablation and replacement happen simultaneously, which is an important safety feature. Hopefully, with these rapid developments, the next generation of retina and genetic medicine specialists will be inspired to advance these technologies and the treatment of inherited disorders.

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR ADVANCED THERAPIES

SPOTLIGHT

EXPERT INSIGHT

Bayesian phase 1/2 trial designs and cellular immunotherapies: a practical primer

Jordan Gauthier, Ying Yuan & Peter Thall

Bayesian phase 1/2 trial designs remain underused in biomedical research and are virtually absent from the field of cellular therapy. In this review, we highlight the severe limitations of the maximum tolerated dose (MTD) concept and the traditional phase 1/phase 2 paradigm. Next, we introduce statistical concepts underlying most adaptive Bayesian trial designs. We use the EffTox design [1,2], one of many adaptive Bayesian designs, as an example to illustrate 'state-of-the-art' phase 1/2 designs. We highlight how these designs can be helpful to the cellular therapy field specifically. Furthermore, we provide the reader with practical examples, links to freely available web applications, and R packages. We hope this will incentivize investigators to implement these designs for chimeric antigen-receptor-engineered T cell therapy trials, as well as other T cell-based therapies.

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INTRODUCTION

Despite the development of many novel clinical trial designs over the past three decades, Bayesian phase 1/2 designs, which hybridize conventional phase 1 and phase 2 designs, remain underused in biomedical research. Reflecting a recent surge of interest in innovative

trial designs, the FDA recently launched a Complex Innovative Trial Designs Pilot Program [3], and issued a draft guidance document entitled 'Adaptive Designs for Clinical Trials of Drugs and Biologics' [4]. In this review, we first will highlight severe limitations of the maximum tolerated dose (MTD) concept and the traditional phase 1/phase 2 paradigm. Next, we will introduce statistical concepts underlying the Bayesian machinery used by most adaptive Bayesian trial designs. Last, we use the EffTox design based on efficacy-toxicity trade-offs [1,2], one of many adaptive Bayesian designs [5–11], as an example to illustrate 'state-of-the-art' phase 1/2 designs. We define the key EffTox design parameters and explain how to interpret trial simulations done using freely available software. While Bayesian I/II designs are more complex than traditional 3+3 algorithms, we believe they are driven by concepts that can be grasped easily by clinicians and researchers. Although we focus on the EffTox design as an example, our goal is to popularize the whole family of Bayesian phase 1/2 designs. We wish to make their underlying concepts more accessible, and to encourage researchers in the field of cellular immunotherapy to use them.

LIMITATIONS OF THE MTD CONCEPT & THE TRADITIONAL PHASE 1/ PHASE 2 PARADIGM

The traditional paradigm splits early clinical drug development into two successive phases: phase 1 trials, to determine the MTD, and phase 2 trials, to evaluate the efficacy of the dose defined as the MTD. Phase 1 trials often are followed by an expansion cohort, in which additional patients are treated at the MTD. Conventionally, most expansion cohorts are devoid of any experimental design, in particular without statistical justification for the sample size [12]; they often generate confusion when unexpected toxicities are observed at the previously selected MTD [13]. Expansion cohorts are still used quite commonly, notably in trials of chimeric antigen receptor-engineered T (CAR-T) cell therapy [14,15].

Many phase 1 studies in oncology rely on so-called '3+3' algorithms [16]. The main advantage of 3+3 algorithms is that they do not require a computer program or a statistician to implement. This apparent simplicity comes with a heavy price. An example of a 3+3 algorithm is shown in Table 1. In most cases, 3+3 algorithms generate unreliable estimates of the true probability of toxicity at each dose (Table 2). When compared to alternative designs, including EffTox, 3+3 algorithms are far less likely to choose a truly optimal dose [17]. Another major drawback of 3+3 algorithms is that they leave many decisions to be made solely, and subjectively, using clinical judgement.

A key assumption underlying the notion of MTD and 3+3 algorithms is monotonicity. This says that a higher dose is necessarily associated with both higher toxicity and higher efficacy probabilities. This motivates the common practice of finding the highest dose with 'acceptable' toxicity, called the MTD. We will highlight limitations of the MTD paradigm by considering several scenarios that are obvious simplifications of more complex biological processes; the dose-toxicity and

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TABLE 1

Example of a phase 1 protocol 3+3 algorithm.

Number of patients with a DLT at a given dose level	Escalation decision rule
0/3	Enter 3 patients at the next dose level
1/3	Enter at least 3 more patients at this dose level If 0 of these 3 patients experience a DLT, proceed to the next dose level If ≥1 of this group suffer DLT, this dose exceeds the MTD and dose escalation is stopped. Three additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose

MTD: the highest dose at which no more than 1 of 6 evaluable patients has had a DLT. Six patients should be treated before the dose is declared as MTD.
DLT: Dose-limiting toxicity; MTD: Maximal tolerated dose.

dose-efficacy relationship shapes can be sigmoid, U-shaped, dome-shaped, and more complex shapes are also possible. Many factors related to cellular immunotherapies – such as patient and disease-related variables, cell product characteristics, *in vivo* kinetics – may alter these relationship shapes. Another advantage of most Bayesian phase 1/2 designs, such as EffTox, is that they make limited assumptions as to the shapes of these relationships. A key point is that the MTD concept overlooks the fact that the relationships between dose and toxicity, and dose and efficacy, may differ significantly, as it is often expected with cellular therapies. This leads to the concept that, among two or more acceptable doses, some might be more desirable than others, for instance when the gain in efficacy is large while the increase in toxicity is small. Let us consider a target toxicity rate of 0.3, which is a commonly used threshold value. In the scenario depicted in Figure 1, toxicity nearly plateaus at dose 4 with a very slight increase from dose 4 to dose 5. In contrast, we observe a sharp increase in efficacy from dose 4 to dose 5. In this scenario, the gain in efficacy clearly outweighs the risk of toxicity. In this case, choosing dose 4 over dose 5 would be detrimental for patients, leading to underdosing in terms of efficacy with comparable toxicity. A flaw with the monotonicity assumption is also seen in a less favorable scenario, where the experimental agent is toxic but has very low efficacy (Figure 2). In this case, no dose is acceptable, but a 3+3 algorithm still will choose a MTD. To relax these oversimplifications, later in this review we will explore the concept of risk–benefit tradeoff utilized in the EffTox design. Of note, 3+3 designs are known to be outperformed by many other phase 1 designs, such as continuous reassessment methods [18], and modified toxicity probability interval (mTPI) designs [19,20].

The biological properties of immune cells are very distinct compared to conventional cytotoxic agents or antibodies, setting them apart from the conventional rules of pharmacokinetics. For example, using a mixed-effect model Stein *et al.* [21] did not observe any relationship between the dose of tisagenlecleucel

TABLE 2

Example of DLT estimates of a simulated phase 1 trial using the 3+3 algorithm showed in Table 1.

Dose	Number of patients	Number of DLTs	95% CI* of the true probability of DLT
1	3	0	0.00–0.71
2	6	1	0.00–0.64
3	3	2	0.09–0.99

*Confidence intervals were computed using the Clopper and Pearson method.
CI: Confidence interval; DLT: Dose-limiting toxicity.

– an FDA-approved CD19-targeted CAR-T cell product for pediatric and young adults with relapsed or refractory acute lymphoblastic leukemia (ALL) – and the Cmax, nor any other model parameter. In contrast, the Seattle group reported a strong association between the dose of JCAR014 and the *in vivo* CAR-T cell expansion in lymphoma patients [22], and to a lower extent in adult ALL patients [15]. The fundamentally different nature of cellular therapies compared to other anti-cancer agents calls for designs capable of capturing various dose–effect relationships without relying on stringent assumptions. More, e.g., a higher dose, might not be necessarily better, e.g., more effective, while being associated with significantly higher toxicity. In this situation, Bayesian phase 1/2 designs such as EffTox, which do not assume linearity and monotonicity, can help us better understand and estimate the shape of the dose-response and dose-toxicity relationships, and identify the dose maximizing efficacy while minimizing toxicity.

STATISTICAL CONCEPTS

Prior & posterior probabilities

Bayesian statistics is based on the probabilistic principle that our future beliefs (e.g., about a treatment effect) are a consequence of our current beliefs (prior probabilities) and newly gathered evidence (likelihood function). This probability relationship, known as Bayes' Law, can be simplified as follows:

$$\text{Posterior probability of outcome (toxicity or efficacy)} \propto \text{Likelihood} \times \text{Priors}$$

The posterior probability of an outcome of interest, given the observed data and our prior beliefs (priors), is a product of the likelihood (how likely the data are for each parameter value) and our prior beliefs. Consequently, particular care should be taken when defining priors in collaboration with statisticians, biologists, and clinicians. Priors reflect both prior beliefs (prior means, or 'most likely probabilities' that are easily elicited from investigators) and prior uncertainty (prior variance or standard deviations that have no intuitive meanings, often determined through computer simulations). Several approaches are possible to construct these priors, which are beyond the scope of this review [2,13,23]. One way of eliciting 'consensus' prior means, is to simply average the probabilities obtained from several experts or investigators. Examples of prior means are shown in Table 3. In practice, priors

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and other design parameters are calibrated by assessing the performance of the design across a broad range of scenarios where the true probability of toxicity and efficacy are specified. Extreme care should be taken running these computer simulations to detect and avoid designs with 'pathological' behaviors, such as the failure to detect the optimal dose with high probability, or the dose finding procedure getting stuck at a suboptimal dose.

Application to adaptive decision-making

Under this framework, decisions to modify the treatment regimen (or simply the dose) are based on posterior probabilities computed under the Bayesian model. Let Dosen denote the dose assigned to the nth patient. This Bayesian sequential decision process can be described as follows:

$$Dose_n \rightarrow Data_n \rightarrow Posterior_n \rightarrow Dose_{n+1} \rightarrow Data_{n+1} \rightarrow Posterior_{n+1} \rightarrow \ldots$$

The outcomes observed after n patients (Datan) are used to compute new posterior probabilities. The decision to repeat or alter the next dose or regimen for the (n+1) th patient (Dosen+1) is based on these posterior probabilities. Thus, by repeated applying Bayes' Law, one may sequentially adapt decisions by using new data to obtain updated posterior probabilities. This often is done after a new cohort of three patients has been treated and evaluated. In the next section, we will specify the process of defining posterior probability criteria for decision-making in the context of the EffTox design.

DESCRIPTION OF THE EFFTOX DESIGN

Dose acceptability

While a number of Bayesian phase 1-2 methods have been developed, we will focus here on the EffTox design, developed by Thall *et al.* [1] and refined by Thall *et al.* [2]. This phase 1/2 Bayesian design has already been used successfully in several clinical trials [24–26]. Free software for trial design and implementation is available at https://biostatistics.mdanderson.org/SoftwareDownload/. EffTox requires the specification of two criteria defining a dose as 'acceptable'. A dose is considered acceptable if, given the current data, there are reasonably high posterior probabilities that

1. the efficacy probability is above a pre-defined threshold; and
2. the toxicity probability is below a pre-defined threshold

FIGURE 1

Favorable scenario where the monotonicity assumption is broken.

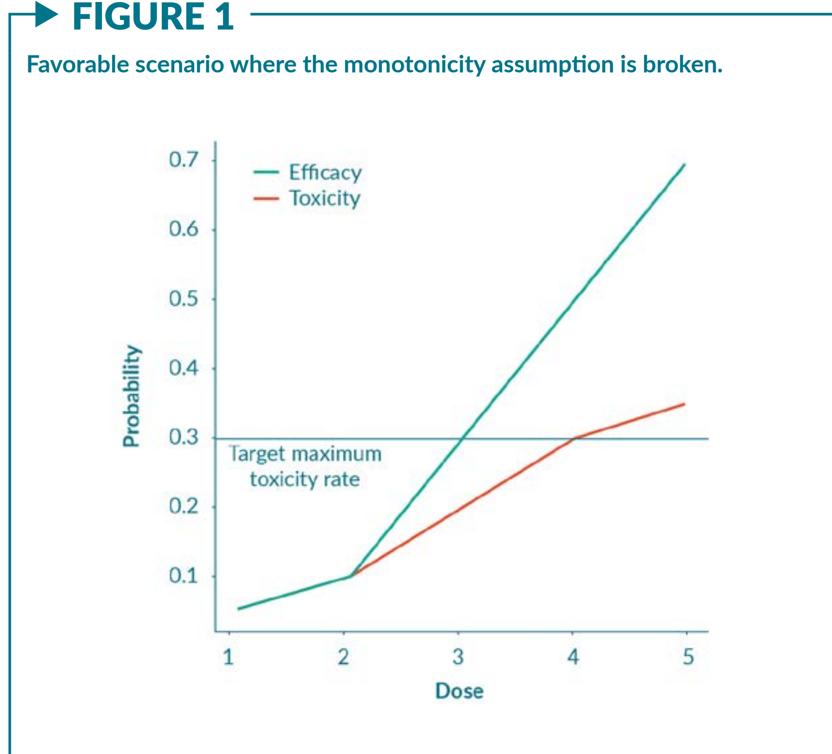
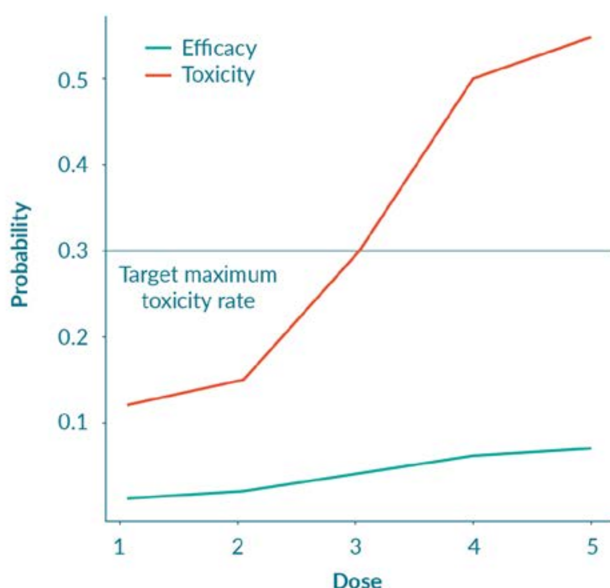


FIGURE 2

Unfavorable scenario where the monotonicity assumption is broken.



These acceptability thresholds are defined by the clinical investigators.

For example, one can consider a dose unacceptable – and conversely that it is acceptable – if either of the following conditions is satisfied:

- There is a ≥ 90% posterior probability that the toxicity probability is > .30;
- There is a ≥ 90% posterior probability that the efficacy probability is < .50. Only acceptable doses are given to patients. If no dose is acceptable, the trial is stopped and no dose is selected.

Dose desirability

Beyond being acceptable, some doses may be more desirable than others. This concept of desirability is the cornerstone of the EffTox design. We consider doses as more desirable when they maximize an objective function that quantifies the trade-off between efficacy and toxicity. To construct this function, efficacy-toxicity trade-off contours are constructed based on three equally desirable pairs of Pr(efficacy) and P{r(toxicity). For example:

1. The probability of efficacy in the absence of toxicity is .40;
2. The probability of toxicity is 65% and the probability of efficacy is 100%;
3. An optimal pair of efficacy/toxicity probabilities equally desirable to 1. and 2. E.g., Pr(efficacy) =.70 with Pr(toxicity) =.25.

Efficacy–toxicity trade-off contours allow us to visualize the optimal trade-offs (Figure 3). The desirability trade-off increases as the pair [Pr(efficacy), Pr(toxicity)] moves from the upper left to the lower right corner of the contour plot. The shapes of the contours help quantifying the trade-off: two dots – two pairs of toxicity/efficacy probabilities – located on the same contour being equally desirable. While the trade-off contour can be difficult to understand and interpret, we have shown that for the EffTox design to perform well, this contour has to be steep [2]. We acknowledge that defining these efficacy-toxicity trade-off values can be challenging in practice; they might also evolve over time. Utility-based designs [8,27], which quantify risk–benefit trade-offs, can in part address this limitation.

Validation through simulations

Before one can use the design for trial conduct, one must carry out a

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TABLE 3

Examples of prior mean probabilities (priors) elicited from the investigators.

	Elicited mean prior probabilities of toxicity	Elicited mean prior probabilities of efficacy
Dose 1	0.02	0.20
Dose 2	0.04	0.40
Dose 3	0.20	0.60
Dose 4	0.50	0.70
Dose 5	0.80	0.80

large number of simulations (usually 1,000) under each of several distinct scenarios (usually 5–10) to ensure the design performs well. A design that performs well has high probabilities of choosing doses that are truly optimal, and high probabilities of stopping the trial early in the unfortunate scenarios where no dose has both acceptable efficacy and toxicity.

The scenarios should reflect a range of possible cases, for example:

- Best scenario: high efficacy, low toxicity at the best dose;
- Worst-case scenario: low efficacy, high toxicity at all doses;
- Middle ground: intermediate efficacy, intermediate toxicity at the best dose;
- Non-linear effects: Pr(toxicity) plateaus, Pr(efficacy) keeps increasing;
- Non-linear effects: Pr(efficacy) plateaus, Pr(toxicity) keeps increasing;
- Nothing happens: low toxicity, low efficacy at all doses.

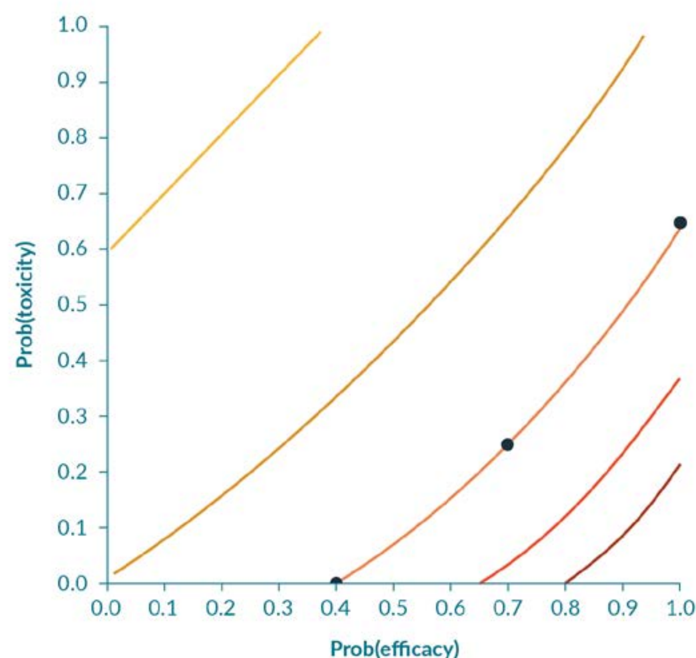
This is not by any means an exhaustive list and it is crucial that investigators, pharmacists, biologists, and statisticians consider all plausible and relevant scenarios, based on the available data, in particular preclinical data from animal models.

Trial conduct

During the trial, a Bayesian adaptive decision process – as described above – is used to determine the optimal dose for each successive cohort, relying on the prior probabilities of efficacy and toxicity

FIGURE 3

Efficacy-toxicity trade-off contours.



The target contour of the function δ is defined by three efficacy-toxicity probability pairs, initially elicited from the investigator, that are considered to be equally desirable. These points are plotted on the graph with blue dots and connected by an orange line representing the target contour. This plot was was generated using free software available at https://biostatistics.mdanderson.org/SoftwareDownload/.

determined by the investigators and the statisticians. The mean posterior probabilities of efficacy and toxicity associated with each dose are computed, and efficacy/toxicity trade-off values (reflecting each dose's desirability) are determined. The next cohort will be given a dose both satisfying the acceptability criteria and having the highest desirability. If no dose is acceptable, the trial will be stopped early with no dose chosen.

APPLICATIONS TO TRIALS OF CELLULAR IMMUNOTHERAPY

Despite a broad panel of design options, Bayesian phase 1/2 designs have been rarely used in trials of immunotherapy. Some examples of Bayesian designs can be found in a limited number of vaccination trials [28,29], although not concurrently evaluating toxicity and efficacy. Some on-going CAR-T cell therapy phase 1 trials rely on the mTPI approach [12,30]. An on-going trial of CAR NK cell therapy [31] is the only one in this field, to date and to our knowledge, using a Bayesian phase 1/2 design.

Trials of cellular immunotherapy pose unique challenges. First, the enrollment potential of these trials is also limited; they are indeed often logistically challenging, extremely costly, and these therapies are currently restricted to selected malignancies in the relapsed/refractory setting. Furthermore, most cellular therapies are only available in a limited number of centers. Taken together, these high costs and relatively small numbers of patients challenge our ability to prospectively address key questions for the field, such as the impact of prior therapies, tumor burden, disease subtype, lymphodepletion, and variables related to the manufacturing of the cellular therapy product (e.g., immune cell phenotypes, *in vitro* T cell functionality). Moreover, cellular therapies such as CD19-targeted CAR-T cell therapy can be associated with severe toxicities, namely cytokine release syndrome and neurotoxicity [32,33]. This highlights an urgent need to inco rporate novel statistical tools to help design and analyze cellular immunotherapy trials. Future research should also aim at comparing the cost-effectiveness of phase 1/2 designs to the conventional paradigm (phase 1 with an expansion cohort, or phase 1 followed by a phase 2 trial).

The following section highlights some of the advantages of phase 1/2 Bayesian trials over conventional 3+3 algorithms:

- Higher probabilities of choosing the optimal dose [17];
- By simultaneously assessing efficacy and toxicity, phase 1/2 designs could dramatically accelerate the development of cellular therapies;
- Evaluating toxicity as well as efficacy concurrently provides significantly more information than a conventional phase 1 trial, which ignores efficacy, thus leading to better estimates of the dose–effect relationships;
- The acceptability criteria reduce the number of patients potentially treated at inacceptable doses, e.g. if toxicity is too high or efficacy is too low.

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REMAINING CHALLENGES

The efficacy and toxicity of cellular therapies, in particular CAR-T cell therapies, are not only functions of the dose administered, they also are strongly dependent on the *in vivo* expansion and persistence of the infused cells. The biological properties of immune cells are very distinct compared to conventional cytotoxic agents or antibodies, setting them apart from the conventional rules of pharmacokinetics. Specifically, T cell functionality [34,35], lymphodepletion [15,22,36], tumor burden [15,37] and expression levels of the target antigen [38,39] are known to dramatically impact the *in vivo* kinetics of CAR-T cells, which subsequently alter the risk of toxicity. These marked variations in the risk of toxicity, for example based on tumor burden, should be taken into account and more complex phase 1/2 designs have been developed to account for prognostic covariates [40]. The limited enrollment potential of cellular immunotherapy trials prompts the field to maximize information gain from trial data; although EffTox was designed to assess only two binary outcomes, new Bayesian phase 1/2 designs have been developed to evaluate multiple co-primary endpoints while accounting for prognostic covariates [40]. One of such designs is used in for a trial of CAR NK cell therapy currently on-going at the MD Anderson Cancer Center [31]. This design evaluates five co-primary outcomes (time to severe toxicity, cytokine release, syndrome, disease progression or response and death) across six prognostic subgroups characterized by the disease type and tumor burden.

One major impediment to adopt novel phase 1/2 designs is that each decision of dose escalation/de-escalation requires real-time, often complicated, model estimation and computation, which can be logistically challenging. This issue has been addressed on several fronts. Freely available, user-friendly software and web applications (https://biostatistics.mdanderson.org/SoftwareDownload/ and http://www.trialdesign.org), as well as commercial platforms (https://udesign.laiyaconsulting.com/), can facilitate real-time model parameter re-estimation and decision making. For R users, several packages have also been implemented to facilitate the use of Bayesian phase 1/2 designs (e.g., trialr, EffToxDesign, dfcomb). Moreover, newer phase 1/2 designs, such as the U-BOIN (utility-based Bayesian optimal interval) design [27], remove the requirement of complicated model estimation. U-BOIN's dose escalation and de-escalation rule can be pre-tabulated and included in the protocol prior to the trial conduct, making its implementation as simple as the 3+3 design, while yielding competitive performance.

We anticipate combinatorial approaches, for example CAR-T cells combined with immune checkpoint inhibitors [30,41] or other molecularly targeted agents [42,43], to become an area of intense focus in the near future. Many phase 1/2 designs are available to optimize two-agent combinations [44,45], or the dose and schedule of administrations [46], accommodate late-onset events [47] and immune response [48], and account for patient genetic heterogeneity [18]. The U-BOIN design can use immune biological outcomes, such

as a prognostic immune biomarker, to predict delayed outcomes [27]. These novel designs can help advance the field of cellular therapy and should be considered when planning early phase trials.

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR ADVANCED THERAPIES

SPOTLIGHT

EXPERT INSIGHT

Considerations for patient selection for cell and gene therapy trials using tumor associated antigens as target in early phase development

Stephanie Traub & David Edwards

For cell and gene therapies, including those using tumor associated antigens (TAAs) as targets, effective patient selection is critical for success. In this paper, we discuss considerations for patient selection for cell and gene therapy products in early phase clinical development. Surprisingly, many obvious key factors like the TAA themselves, the major histocompatibility complex (MHC), as well as practical implication of patient selection on the trial design and conduct are not given the consideration that they should be given. The article focuses on ideal patient selection for cell and gene therapies using TAAs and implications for clinical trial design.

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CELL & GENE THERAPIES USING TUMOR ASSOCIATED ANTIGENS

TAAs are attractive anti-cancer targets. Where tumor specificity is optimized to reduce off-tissue effects, it can reduce many of the side effects commonly observed with more conventional therapies

[1]. CAR T-cell therapies and TCR T-cell therapies utilize TAAs to attack the tumor directly; and indirect vaccination approaches like autologous and allogenic dendritic cell (DC) therapies use TAAs to induce antigen-specific T cells.

TUMOR ASSOCIATED ANTIGENS

Less than 100 TAAs have been identified so far and some of them tested in clinical trials [2,3]. The key factor for a good antigen target is tumor-specificity, i.e., an antigen which is ideally not expressed in other tissues. In reality, this is rarely the case: most TAAs are expressed in tissues other than tumor. What is of importance, however, is that TAA expression is higher in the tumor than in the other tissues. For example, prostatic acid phosphatase (PAP) has been found to be expressed in the prostate. PAP is not restricted solely to prostate tissue, but its expression in other tissues is ~1–2 orders of magnitude less than that observed in the prostate [4]. Specifically, when targeting a new unknown antigen which has been not intensively explored, it is of importance to check for tissue expression in addition to its expression on tumor tissues. TAA selectivity is important; there have been cases where unexpected toxicity has occurred due to recognition of an epitope from an unrelated protein [5].

An additional consideration is the degree of heterogeneity that the TAA displays; ideally, every tumor cell would express the TAA. In some cases, such as NY-ESO-1 (New York-esophageal squamous cell carcinoma-1) expression in hepatocellular carcinoma, not every tumor cell expresses it. Instead, TAA positive cells display a patchy appearance and this might limit the efficacy of any TAA targeted therapy [6]. When selecting a TAA for a cell and gene therapy product, the selectivity of the therapy and the heterogeneity of the antigen in the tumor need careful consideration to minimize off-target toxicity and optimize efficacy.

MAJOR HISTOCOMPATIBILITY COMPLEX

The major histocompatibility complex (MHC), also called HLA (human leukocyte antigen) complex, is a set of genes that are co-dominantly expressed and are highly polymorphic [7]. MHC class one (MHC-I) and two (MHC-II) proteins are expressed on APCs as well as B lymphocytes and MHC-I on almost all nucleated non-APCs [8,9].

The inheritance of the HLA haplotypes from each parent results in a random combination of different HLA loci. However, some HLA haplotypes are over-represented in certain populations: HLA-A1, -B8, -DR17 is the most common HLA haplotype among Caucasians [8]; the subtype genotype HLA-A*02:01, is found in different frequencies in populations, e.g., in Finland, HLA-A*02:01 would be found in 34.4% of the population while in Thailand, 1.8% would have the genotype [10,11]. It might be that regional differences due to HLA prevalence can occur and impact patient selection. The implications of this variability need to be carefully considered both when designing MHC restricted therapies for a particular region and when deciding where to locate clinical trial centers that will efficiently recruit.

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The predicted abundance of the MHC allele in the population needs to be factored in when considering eligibility screening; underestimation can impact on time lines and budget.

TAA & HLA

Prevalence of TAA

There is often discordance between the prevalence of tumor antigens described in the literature and that encountered during trial screening. Literature data sets are often relatively small with limited numbers of cases of tumor samples. In addition, protein data via immunohistochemistry (IHC) or expression data via RT-PCR (reverse transcriptase polymerase chain reaction) might have been taken into consideration, which could give differences in results. Additionally, the method of the IHC retrieval system might give higher antigen numbers in certain cases [12]. Another potential confounder occurs if the stage of disease in the trial patients differs from that of the reference population. For example, for MAGE-A3 (Melanoma Antigen Gene A3) 31 out of 105 (29.5%) stage I non-small cell lung cancers and 49 out of 99 (49.5%) stage II non-small cell lung cancers expressed MAGE-A3 [13]. In clinical practice, e.g. in the MAGRIT trial stage IB, II and IIIA NSCLC patients have been recruited and 33% had MAGE-A3 positive tumors (4210/12820) [14] (ClinicalTrials.gov Identifier: NCT00480025). Therefore, it may be prudent to generate this data in support of a trial using biobank material, especially when selecting for less well known TAAs to estimate patient recruitment requirements.

A second aspect to consider is the actual failure of the analytical method or issues around the sample given by the patient. In the MAGRIT trial 7.4% of patient screened did sign the consent but no sample was sent, insufficient tumor sample was available, contaminations in the analytical method did not give a result, and there were other issues. In addition, even after being identified as MAGE-A3 positive, a large number of patients (45%, 2312/4210) did not meet the eligibility criteria – e.g. patients variously not having signed informed consent for main study, not being found to be free of metastasis, having concurrent severe medial problems and many more [14] (ClinicalTrials.gov Identifier: NCT00480025). Logistical considerations can also impact on the efficiency of patient recruitment; slot availability can be a significant issue. In the absence of a treatment slot, clinical sites are not motivated to screen patients; even if patients are available, they may progress before they can be recruited or move on to other treatments.

To conclude, TAA prevalence impacts the efficiency of trial recruitment. To ensure that a trial can efficiently recruit, the number of centers open to recruitment should reflect the frequency of the TAA in the patient population. Secondly, the trial should be designed to avoid unnecessary delays to recruitment; for example, expedited trial escalation decisions can help increase the recruitment speed.

TAA presentation in HLA

Foreign- and self-peptides are presented to the cells of the immune system through the MHC system.

This presentation takes place in the groove of the MHC-I for endogenous and intracellular peptides, and for exogenous and extracellular peptides the presentation takes place in the groove of the MHC-II. Through cross-presentation, exogenous antigens can be presented by MHC-I and endogenous antigens can be presented by MHC-II when they have been degraded by autophagy [15]. Due to the properties of the MHCs, only specific peptide epitopes can be presented; length and structure play a major role for the capability of a peptide epitope to be presented. Consequently, not all of the possible antigen fragments are equally presented on the cell surface. Furthermore, of the antigens that are presented, not all are equally immunogenic. As an example, Table 1 illustrates some of the antigens that can be obtained from NY-ESO-1 and their relative immunogenicity. Clearly, not all TAAs induce strong immunogenicity and the T cell response can be destructive and non-destructive for the tumor [16]. When developing a therapeutic using TAA, the choice of appropriate epitope selection is essential if the intervention is going to induce highly immunogenic and tumor destructive T cells.

PATIENT SELECTION FOR CELL THERAPY

Autologous & allogeneic dendritic cell therapy

TAA match with cell therapy is of importance, as without the tumor expressing the antigen no specific immune response will be possible. One successful example of a cell therapy using patient selection is the first FDA-approved autologous cellular immunotherapy, Sipuleucel-T, which uses autologous antigen presenting cells cultured with a fusion protein consisting of PAP (prostatic acid phosphatase) linked to granulocyte-macrophage colony-stimulating factor. In the Phase 3 trial, patients with positive IHC staining of PAP in at least 25% of cells were eligible for trial entry [17].

Several approaches to generate allogeneic DCs have been taken – for example, DCs generated from cord blood [18] have been tested in the clinic (ClinicalTrials.gov Identifier: NCT01373515) [19]. Another trial used embryonic stem cell-derived DCs [20], which were tested in a clinical trial in a confirmed HLA-A*02:01 positive population to guarantee at least one HLA match with each receiving patient's immune system (ClinicalTrials.gov Identifier: NCT03371485). However, these allogeneic cells must be HLA matched to the recipient to ensure that the DC can interact with host T cells in a productive way and the DC is not rejected by the host. In the case of autologous DCs, patient selection should be considered for the TAA; for allogeneic DCs, patient selection should ideally consider selection for TAA and match for HLA.

TCR-T cell therapy & CAR-T cell therapy

Another potentially promising form of cell therapy is T-cell receptor engineered T-cells (TCR-T). TCR-T rely on the interaction of peptide:MHC, formed by peptide bound to MHC. To effectively kill, the T-cell receptor must be matched with at least one HLA allele from

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TABLE 1

NY-ESO-1 epitope sequence, HLA presentation and immune response.

MHC class	Allele	Epitope sequence	Immune response	Ref.
I	HLA-A2	$SLLMWITQCFL_{157–167}$	NY-ESO-1-specific CD8+ T cells, some stabilization of disease, and regression of individual metastases in some patients	[9]
I	HLA-A2	$SLLMWITQC_{157–165}$	Very efficiently recognized by CD8+ T cells from HLA-A*0201 melanoma patients and epitope identified in patients with spontaneous immunity	[34–36]
I	HLA-A2	$QLSLLMWIT_{155–163}$	Is poorly immunogenic and CD8+ CTLs recognizing this epitope are rarely detected in cancer patients	[37–39]
I	HLA-A2	$LMWITQCFL_{159–167}$	Not naturally processed	[34]
II	HLA-DPB1*0401–0402	$SLLMWITQCFLPVF_{157–170}$	CD4+T cell responses were induced in a high proportion of patients	[40–42]

This list is not inclusive of every epitope sequence that can be possibly presented, and only the principle of the relationship of epitope processing and presentation is illustrated.
Colour indicates identical amino acid sequence in peptides.

the patient [21]. One of the technologies is Adaptimmune's SPEAR T cells (ClinicalTrials.gov Identifier: NCT04044859), where patient selection is done and eligibility for the trial requires either positivity of HLA-A*02:05 or HLA-A*02 allele, and tumor must show confirmed MAGE-A4 expression. Therefore, for effective therapeutic effect, TCR-T therapy trials should ideally select for TAA and HLA match.

CAR-T cell therapy has made great progress in CD19⁺ hematological malignancies [22] although recent trials in solid tumors have failed to replicate these initial success. CAR-T therapies targeting antigens like EGRFvIII, IL-13Rα2, HER2, EphA2 and GD2 are in clinical development [22]. CAR-T cells do not require selection for HLA, as they are independent of MHC presentation. The binding works via an antibody-derived domain for binding with the TAA and the intracellular part is that of a TCR-derived signaling moiety for T cell activation. Therefore, recognition and activation is independent of MHC presentation, binding is defined only by the antibody domain [23]. No selection for MHC is necessary, but selection for the presence of the TAA on the tumor cells would be of prime importance for CAR-T therapy.

HLA general considerations

HLA genotypes are involved in the response to treatment; certain HLA genotypes are associated with a more favorable response than others [24]. Retrospective analysis of patients treated with immune checkpoint inhibitor showed improved overall survival in patient with HLA-B44, whereas HLA-B*15:01 might impair T cell recognition of neoantigens [24]. Others have shown that HLA variations might be associated with adverse events to checkpoint inhibitors [25], and patients with a

specific genotype are more likely to develop side effects due to their genotype. There is a clear therapeutic rationale to test cell and gene therapies in combination with checkpoint inhibitors and under these circumstances, consideration should be given to the effect of the genotype on the combination therapy. The ideal HLA genotype for the cell and gene therapy might not be favorable for the combination agent.

Practical implications for TAA & HLA selection

Patient selection is a powerful tool to help ensure that maximum patient benefit can be achieved. However, tissue based TAA selection requires the availability of an adequate amount of suitable tissue making tumor accessibility a key consideration; inaccessible tumors may not be readily biopsied. Archival tumor biopsies might be considered if stability data are available which show the TAA is stable in FFPE (formalin-fixed paraffin-embedded). Also, data should be available that the TAA expression is not altered by previous or ongoing treatment during which the archival tumor biopsy has been taken. Additionally, the biopsy itself has an intrinsic risk factor associated, especially in difficult to access tumor locations like the lung. Increased risk is associated with the lung biopsy, e.g., pneumothorax and bleeding [26].

The method of choice for TAA selection is IHC when the protein of interest is expressed on the cell surface, or RT-PCR when either there is no specific antibody available to detect the TAA in FFPE tissue or the TAA is only expressed intracellularly. RT-PCR samples are rarely generated from FFPE tissue, but from snap frozen biopsies or biopsies stored in RNA stabilizing reagents. The latter two require fresh tumor biopsy, which places further constraints on trial design and logistics. For IHC assessment of TAAs, a central lab might be preferred over a local lab as local differences in staining procedures, cut-off assessment impacts upon the positivity rate. If the use of local labs is unavoidable, this can be solved by circulating positive tissues between local labs and getting concordance between the pathologist doing the readout of the staining. HLA selection can be done conveniently from peripheral blood with which no issues with availability of tissue sample are normally encountered.

HLA loss on tumors & other mechanisms of tumor disguise

Tumors are adept at hiding from their host's immune system by utilizing numerous mechanisms; one such mechanism that leads to tumor resistance to therapy [27] is tumor cell MHC-I loss or downregulation to avoid recognition and elimination of T lymphocytes [28]. Reduced HLA expression as well as HLA loss of heterozygosity by cancer cells helps cancer cells escape and avoid cytotoxic T lymphocytes [29,30]. As well as reducing the abundance of antigens expressed on the cell it is also possible that proteins can be processed differently in cancer compared with healthy cells resulting in the presentation of unique antigens in MHC-I [31,32]. These aspects have been underexplored regarding patient selection until now. It might be worth considering including in

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patient selection when the cell and gene therapy is MHC dependent. If there is a requirement for HLA engagement by a cell and gene therapy drug product, selection for presence of MHC-I on tumor cells might be considered.

Other mechanisms of acquired resistance include defects in antigen processing and presentation resulting in the loss of peptide presentation in the MHC complex. In addition, loss of immunogenic antigens in general by the tumor can occur; peptides of the tumor are displayed in the MHC:peptide complex, but no immunogenic peptides remain and immunogenic response against the tumor is abrogated [33].

CONSIDERATIONS FOR CLINICAL TRIAL DESIGN FOR EARLY PHASE STUDIES

Typically, Phase 1 trials focus upon the safety of the investigational drug product. However, efficacy assessment and understanding the mechanism of action to aid development decision-making is becoming more important. The effort required to recruit adequate numbers of potentially responsive (correct TAA expression and HLA genotype) patients even for a small trial should not be under estimated. To maximize the chance of success, careful consideration should be given to the accuracy of any literature data on TAA prevalence; generation of pilot data should be considered. Flexible cohort size and flexible trial design will minimize lack of slot availability. Consideration should be given to how to incentivize trial sites to continually screen patients. Patients can also fail eligibility on other exclusion criteria or progress during screening, and these failures need to be factored in when considering optimal screening rates. Patient selection can be complex, and a balance must be struck between recruiting individuals who are likely to respond and have benefit from the treatment, and the clinical feasibility of the trial. Screening is a major but often overlooked hurdle to successful translation, but when considered carefully, it can lead to fruitful clinical outcomes.

TRANSLATIONAL INSIGHT

Considerations for patient selection for cell and gene therapy trials using tumor associated antigen as target in early phase development:

- Selection of the right TAA taking into consideration the immunogenicity of the TAA, selectivity and specificity, and the heterogeneity in the tumor
- Prevalence of the TAA and the resulting impact on patient recruitment
- Ideal selection strategy for autologous DCs and CAR-T cell therapy should include selection for TAA, allogeneic DCs and TCR-T cell therapy should include selection for TAA and HLA
- Selection for loss of HLA on tumors might be considered
- Accessibility of tumor and the resulting choice of indication
- Optimization of trial design to ensure efficient recruitment

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR ADVANCED THERAPIES

SPOTLIGHT

EXPERT INSIGHT

The evolution of adeno-associated virus capsids for CNS gene therapy

Steven J Gray

Adeno-associated virus (AAV) is emerging as a dominant gene therapy delivery vehicle, and the broad tool kit of naturally-occurring AAV capsid variants has allowed tailoring of approaches for specific applications. For example, Glybera® (AAV1) is targeted to muscle, Luxterna™ (AAV2) is targeted to the retina, and Zolgensma® (AAV9) is targeted to the central nervous system (CNS). In the context of CNS gene therapy, the discovery of AAV9 was largely responsible for a shift from direct intraparenchymal brain injections to approaches that more globally target the brain, such as intravenous injection and/or injection into the cerebrospinal fluid (CSF). In fact, one could divide CNS gene therapy into 'pre-AAV9' and 'post-AAV9' eras, due to the dramatic leap that this vector technology enabled. One can envision a similar future leap coming, as lab-derived improvements to capsids are being made that could further increase the efficiency and specificity of CNS-directed gene therapy. Recent advancements in AAV vectors are discussed.

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CNS GENE THERAPY: HOW WE GOT TO WHERE WE ARE NOW

The first CNS-directed AAV gene therapy trials started in the early 2000s, with stereotaxic administration of AAV2 vectors for Canavan disease, Parkinson's disease, and CLN2 Batten disease [1–13].

These approaches were well-tolerated, and localized treatment effects could be seen. However, overall the efficacy of the treatments was limited and generally not deemed to be highly disease-modifying. Other naturally-occurring AAV serotypes were identified and characterized, such as AAV5, AAVrh8, and AAVrh10, which demonstrated greater spread from intraparenchymal injection sites compared to AAV2 [14–16]. As an additional advancement, novel injection parameters such as convection-enhanced delivery were optimized to provide greater and more controlled spread of AAV vectors from a stereotaxic, intraparenchymal injection [17,18]. While these represented improvements in the general approach to CNS gene therapy, they were incremental and typically did not translate into a transformative benefit to patients. Exceptions to this are cases where localized expression of the transgene could be particularly disease-modifying, such as in the clinical trial for aromatic L-amino acid decarboxylase (AADC) deficiency [19]. The AADC clinical trial in particular was groundbreaking, as the clearest demonstration at the time of substantial benefit imparted by an AAV vector for a CNS disease. Following bilateral stereotaxic injection of an AAV2/AADC vector to the putamen, patients gained new motor function with a dramatic improvement on quality of life. On the whole, however, the approach to target an organ the size of a human brain by multiple stereotaxic injections progressed in a very limited and incremental fashion, with treatment of most CNS disorders impractical by this approach.

In 2006, the discovery of dozens of new AAV capsid variants provided a wealth of potentially new vector tropisms to uncover [20]. By 2009-2011, a critical discovery was independently described by three different laboratories that one of these novel capsids, AAV9, was able to cross the blood brain barrier (BBB) [21–23]. Furthermore, the ability of an intravenously-injected AAV9 vector to target the brain was shown in these initial three reports to translate from mice to adult cats and non-human primates. The technology was rapidly adopted by laboratories around the world, with numerous publications demonstrating substantial efficacy in small and large animal models. In contrast to direct intracranial approaches, the translation of dose and vector administration of intravenous AAV9 to large animals and humans was relatively straightforward. As a result, after 5 years the first intravenous AAV9 clinical trial was initiated for Spinal Muscular Atrophy (SMA) in 2014. In 2017 the initial results from the Phase 1 SMA trial were published, documenting unequivocal and unprecedented benefit for a CNS disease from gene therapy [24]. In 2019, the AAV9/SMN1 vector became an FDA-approved drug, termed Zolgensma®. In the meantime, the success of the SMA program prompted many similar AAV9 efforts to move into clinical trials, such as for Mucopolysaccharidosis (MPS) Type IIIA, MPS IIIB, GM1 Gangliosidosis, and Danon disease (clinicaltrials.gov identifiers NCT02716246, NCT03315182, NCT03952637, and NCT03882437, respectively). In short order, intravenous AAV9 became a platform approach to treat multiple CNS disorders in a similar fashion.

It should be noted that intravenous injection of AAV9 has several major limitations, including the high dose

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of vector that needs to be administered (and manufactured) per kilogram body weight, the substantially higher (>100x) biodistribution of the vector to peripheral organs compared to the brain, and the relatively high prevalence of natural antibodies against AAV9 in the human population. It was found that injection of AAV9 into the CSF could overcome many of these limitations, and the intra-CSF approach with AAV9 still translated well from rodents to larger animals [25,26]. Similar to the expanding use of AAV9 in intravenous clinical trials to target the CNS, AAV9 intrathecal trials initiated in 2015 for Giant Axonal Neuropathy, followed by trials for other diseases such as CLN6 Batten disease, MPS I, MPS II, and CLN3 Batten disease (clinicaltrials.gov identifiers NCT02362438, NCT03580083, NCT03566043, NCT02725580, and NCT03770572). Based on the results of the SMA clinical trial and numerous strong preclinical study results across many disease models, the expectation is that the number of AAV9-based clinical trials (intravenous or intrathecal) will continue to expand at an accelerating pace.

LIMITATIONS OF AAV9 & THE NEED FOR BETTER AAV VECTORS

While AAV9 has transformed the field of CNS gene therapy and can mediate transformative treatments for many diseases, it has substantial limitations. As mentioned, if it is administered intravenously it is susceptible to rapid neutralization by circulating anti-AAV9 antibodies. Approximately 17–47% of the human population has detectable neutralizing antibodies against AAV9, due to natural AAV infections [27–29]. These patients would be excluded from trials or treatments utilizing intravenous administration of AAV9. One strategy to overcome these naturally-occurring antibodies would be to utilize lab-engineered capsid variants that would be serologically distinct from natural AAVs.

Another limitation is the biodistribution pattern of the AAV9 vector. When administered IV, less than 1% of the vector is localized to the brain [21]. When administered intrathecally, a large portion of the vector distributes to peripheral organs, with vector genome copy numbers in the liver exceeding that of the spinal cord near the site of injection [25]. Thus, AAV9 lacks specificity or even preferred tropism for the CNS. Related to the biodistribution of the vector, within the CNS AAV9 has been described to target neurons and astrocytes, and to a lesser degree oligodendrocytes and endothelial cells. Although there is some inconsistency in the literature, there are multiple reports that in juvenile or adult primates the AAV9 vector has a preference for astrocytes over neurons especially when administered intravenously [21,22,30].

There are some concerns about potential toxicities using AAV9 vectors. In the SMA clinical trial using IV-injected AAV9 vectors, transient liver toxicity was noted, but it was responsive to steroids and could be managed with a prophylactic steroid regimen [24]. This is consistent with findings seen in other clinical trials administering AAV8 vectors IV such as for hemophilia, where dose-responsive liver toxicity was observed that could be resolved with steroids [31]. A recent publication

from Jim Wilson's laboratory documented dorsal root ganglia toxicity in NHPs and pigs following administration of an AAV9-like variant [32], but this finding has not been corroborated in other similar NHP studies using AAV9. Of greater concern was the rapid death of one of the NHPs following IV administration of the AAV9-like variant. This appears to have been an isolated and unexplained incident that may or may not have been directly linked to the AAV capsid. Regardless, these cautionary potential toxicities could presumably be managed better if a more efficient AAV capsid was available that would allow lower vector doses to be equally therapeutic.

In terms of overall efficiency of CNS gene transfer, ideally a vector would target a majority of affected cells across the CNS. However, with AAV9 there has never been a report of saturating (near 100%) transduction efficiency across the CNS in any animal model, with the exception of studies dosing prenatal or neonatal rodents. Rather, biodistribution numbers are typically below 0.5 copies per cell on average, meaning that large numbers of cells across the CNS are not receiving any vector DNA, let alone stably expressing the transgene. For the sake of discussion, an assumption is made that intravenous doses have a ceiling at roughly 1×10^{14} vg/kg, and intrathecal doses might have a ceiling of a 10 mL injection volume in humans (1×10^{15} vg total); thus, higher transduction efficiency can't easily be achieved simply by increasing the dose. While it is clear from multiple studies that this level of transduction efficiency is sufficient to provide at least some level of therapeutic benefit for many diseases, for most CNS disorders a higher transduction efficiency would be needed to maximize therapeutic efficacy.

BEYOND AAV9: STRATEGIES TOWARD THE NEXT LEAP IN VECTOR TECHNOLOGY

AAV9 is one of over 100 naturally-occurring AAV capsid variants that have been isolated. While it is possible that another naturally-occurring AAV capsid could be identified with superior CNS-targeting capabilities, most efforts to develop a better AAV-based CNS vector have focused on creating novel laboratory-derived AAV capsids. Toward this end, there are 2 main strategies: 1) rational design and 2) directed evolution.

Rational design takes a hypothesis-driven approach to generate AAV capsid variants, utilizing structure-function knowledge about AAV capsid biology. In two independent approaches, strategies were pursued to increase the spread of AAV vectors by knocking out their primary proteoglycan receptor. In the first instance, Albright *et al.* investigated the role of sialic acid binding in the ability of an AAV1 variant (AAV1RX) to cross the BBB and transduce neurons in the CNS [33]. AAV1 normally binds cell surface proteoglycans with terminal sialic acid. When the variable region I from the BBB-crossing rh10 capsid was swapped into AAV1 to create AAV1RX, it allowed AAV1 to cross the BBB while also strongly reducing the dependence of AAV1RX to utilize sialic acid [34]. After testing a variety of AAV1 and AAVrh10 mutants with different levels of dependence on sialic acid

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binding, Albright *et al.* proposed a model whereby fine-tuning of sialic acid binding becomes important for CNS transduction after intravenous administration. Some sialic acid binding was necessary for CNS transduction; however too much sialic acid binding led to high liver tropism and reduced BBB crossing. In the second approach to modify primary proteoglycan receptor binding, Sullivan *et al.* introduced R585A and R588A mutations into AAV2 (AAV2HBKO) to knock out its ability to bind heparin sulfate proteoglycans, the primary receptor for AAV2 [35]. Upon direct intracranial administration into the striatum of mice, AAV2HBKO showed considerably larger transduced areas compared to AAV2, presumably due to lower heparin binding at the site of injection allowing better spread. In a more unorthodox third approach to rational design, the Vandenberghe lab has taken an *in silico* strategy to reconstruct ancestors of modern AAVs. One reconstructed variant, termed Anc80L65, was able to cross the BBB after intravenous administration in adult mice, and transduce approximately 3–4 times the number of neurons and astrocytes compared to AAV9 [36]. Overall, these examples demonstrate the potential of rational design strategies to yield better CNS vectors.

Directed evolution approaches have also shown considerable promise to generate novel AAV capsids with substantially altered properties. These approaches utilize libraries of AAV capsid variants produced by random peptide integration, random mutagenesis, and/or shuffling of multiple AAV capsid sequences. Shuffled capsid AAV variants and lab-engineered AAV capsids can have lower cross-reactivity to antibodies resulting from natural AAV infections [37]. Directed evolution approaches have been utilized to generate novel AAV capsids with greatly enhanced CNS transduction following intravenous administration in mice (PHP.B, [38]), efficient transduction of oligodendrocytes following intracranial injection in rats and non-human primates (AAV-Olig001, [39,40]) and enhanced retrograde axonal transport in mice (AAV2-retro, [41]). While the PHP.B variant of AAV9 confers approximately 50-fold enhanced CNS transduction compared to AAV9 in mice following IV administration, unfortunately this greater CNS transduction does not translate to primates [42–44]. In contrast, the preferred oligodendrocyte tropism and degree of spread of Olig001 after intraparenchymal injection into the striatum does translate between rodents and non-human primates [40]. Thus, while directed evolution has the potential to generate valuable capsid variants with novel characteristics, they have a mixed track record of effectively translating out of rodent models.

EXPERT INSIGHT

Despite considerable effort to derive AAV vectors in the laboratory with superior abilities, and the first use of a non-natural AAV capsid (AAV2.5) in a clinical trial reported in 2010 [45,46], the vast majority of AAV clinical trials still use unmodified capsids that can be found in nature. In the context of CNS gene therapy, AAV9 is the gold standard and has provided the first suggestion of a 'on-size-fits-all' gene transfer approach to treat many CNS

disorders. While this is likely the case for dozens of inherited CNS disorders, the vast majority would benefit greatly from (or outright require) better vector technology. With the tools available to the field, it is likely that a vector will become available that is superior to AAV9 in terms of specificity and/or efficiency. However, at this time there are no published reports demonstrating an AAV capsid more efficient than AAV9 for widespread CNS gene transfer in non-human primates. The gold standard that any new capsid needs to meet is to be tested directly against AAV9 in non-human primates. If a novel AAV capsid is proven to have five- or ten-fold greater transduction efficiency broadly across the CNS compared to AAV9, following an intravenous or intra-CSF injection in non-human primates, we can expect another monumental leap in our ability to treat CNS disorders.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR
ADVANCED THERAPIES

SPOTLIGHT

REGULATORY PERSPECTIVE

Clinical trials of advanced therapy investigational medicinal products in Spain: preparing for the European clinical trials regulation

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Clinical trials (CT) of Advanced Therapy Medicinal Products are a reality worldwide. Although ATMPs are still very innovative therapies, it is interesting to investigate what relevant information can be obtained from the analyses of authorized CT and the investigated products. The aim of this study was to follow the evolution of CT with Advanced Therapy investigational Medicinal Products (ATiMP) authorized in Spain from May 2004 to June 2019 on the basis of information available at the Spanish Agency for Medicinal Products and Medical Devices and their real status (also taking into consideration their status in three different official Registries). We will also discuss how sponsors and Authorities can prepare for the coming new clinical trial regulation and take advantage of the opportunities it may present.

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INTRODUCTION

Clinical trials (CT) are essential to support the authorization of medicinal products and are the basis for their appropriate use in normal clinical practice. The knowledge of ongoing or finished CT is essential in order to favor better designs for future clinical investigations. There is a CT European legislation in force since 1st May 2004 (Directive 2001/20/CE) [1] that has been reviewed in CT Regulation 536/2014 [2]. Under both legislations, the conduct of a clinical trial with a medicinal product in any European Union (EU) Member State requires prior national authorization. In the case of Spain, such authorization is given by the Spanish Agency for Medicinal Products and Medical Devices (AEMPS) after internal CT review, provided that one Ethics Committee (Committee of Ethics of the Investigation with medicinal products – known in Spanish as CEIm) has also given a favorable opinion. The information that sponsors currently need to provide to the competent authorities (AEMPS in the case of Spain) to be published in either EU CT Register (EU CTR) [3] or Spanish Register on Clinical Studies (REec) [4] is shown in Table 1.

The above-mentioned reviewed Regulation came into force in June 2014 and introduced important changes; among them, a European coordinated assessment of CT and additional transparency requirements with respect to terms currently in force and shown in Table 1, related to CT information and documents that will be available to the public [5]. However, its whole applicability is still pending the availability of the new EU CT Portal and Database (CT Information System), which is currently under development and will enormously simplify communications between sponsors and Member States (MS). In the meantime, a Voluntary Harmonisation Procedure (VHP), set up by the Clinical Trials Facilitation Group, serves as a pilot for the coordinated EU assessment of CT applications foreseen in Regulation 536/2014(2). The VHP was introduced in order to achieve harmonized assessments and decisions on clinical trials in the EU, and sponsors are encouraged to use it [6,7].

Advanced Therapy medicinal products (ATMP) are a particularly innovative medicinal class that includes gene therapy medicinal products (GTMP), somatic cell therapy medicinal products (sCTMP), tissue engineered products (TEP), and combined products (tissue or cell associated with a device). The legal and regulatory framework for ATMPs in the EU (ATMP Regulation 1394/2007) [8] came into force on 31st December 2008 and defined common rules for this very innovative group of medicinal products that have to comply with specific quality requirements [9].

Clinical investigation of ATMP has additional difficulties due to the nature of some of the products. For instance, many cell-based ATMPs are autologous (i.e. prepared from material taken from the patient) which makes standardization a real challenge for manufacturers. In addition, Advanced Therapy investigational Medicinal Products (ATiMP) have to comply not only with the general legislations for clinical trials and ATMP, but also with legislation from different frameworks, such as the tissues and cells Directive (Directive 2004/23)

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TABLE 1

Information to be provided by the sponsors to the AEMPS to be published in the EU CTR and REec according to EU and national legislation [2,14].

CT information to be provided by the sponsor to NCA to be public	Publication in EU CTR	Rules for publication of CT in REec
Summary of CT design (since initial CT application for authorization)	All CT authorized since 1st May 2004. However, Phase 1 CT not including pediatric population are not published	All CT authorized since 1st January 2013. Phase 1 CT not including pediatric population may only include abbreviated information, if this is the sponsor choice
Date of CT start (within following 15 days)	Yes	Yes
Date of end of recruitment in Spain (within following 15 days)	No	Yes
Dates of end of CT in Spain and of global CT end, clarifying if the end is premature or not (within following 15 days)	Yes	Yes and in case of premature end, reasons are also published after assessment
Temporary halts affecting Spain clarifying if global or not and reasons (within the following 15 days)	Yes, reasons are not published	Yes, and reasons are also published after assessment
Summary of CT results (within one year of the date of global CT end)	Results to be loaded in EudraCT and also submitted to the AEMPS	Results of Phase 1 CT not including pediatric population are currently not public

for the donation, procurement and testing of the starting materials to be converted into cell-based medicinal products [8], or that for genetically modified organisms (GMO) (Directives 2001/18 and/or 2009/41) [8] when the product belongs to this category. In this latter case, lack of harmonization between different GMO authorities across the EU has prompted the development of common voluntary procedures for some categories of products [10].

In spite of the above difficulties, Spain has been identified as the Member State with the highest number of CT on ATMPs [6,11]; taking advantage of this, we set out to analyze characteristics of these CT. This article is focused on the analysis of the characteristics of the clinical trials on ATiMP authorized by AEMPS from 1st May 2004 to 30th June 2019, also paying attention to the IMP being investigated. The purpose is to identify possible areas of improvement in order to be able to comply with the new EU CT legislation.

METHODOLOGY

All valid clinical trial applications on ATiMP received at AEMPS since 1st May 2004 until 30th June 2019 have been considered for the analysis. Description of the characteristics of the clinical trials authorized by AEMPS takes into account the information available on the internal CT database of this Agency regardless of substantial amendments.

ATiMPs were classified according to the definitions set out in Regulation 1394/2007 and Directive 120/2009, and following the principles highlighted in the reflection paper on classification of ATMPs published by the Committee for Advanced Therapies (CAT) [12]. Products used in clinical trials before these definitions were published have been reclassified according to

these criteria in order to have a harmonized approach. Products containing or consisting on genetically modified cells (e.g., CAR-T cells) are generally considered GTMPs in the EU, except when the genetic modification is not directly linked to the therapeutic activity of the cells.

In Spain, all medicinal products without a marketing authorization in any country of the European Economic Area (EEA) that contain an active substance or combination of substances not included in any of the medicinal products marketed in Spain need to obtain a number of Product under clinical investigation (known in Spanish as PEI) and sponsors need to cross-reference this number for every new CT application. A PEI covers all pharmaceutical forms and strengths of an investigational product. ATiMPs and especially, cell-based ATiMPs, are very complex and sometimes it is difficult to determine whether a particular product should be considered the same or a different PEI. For instance, a different PEI number is required when the same cell product changes from an autologous to an allogeneic use. Normally, when changes were introduced – e.g., in final formulation – the new product was considered as being different. When substantial changes were introduced in manufacturing without a proper comparability study, the final products were also considered as being different. Different manufacturers require different PEI numbers unless equivalence of the products is shown through strong comparability studies. To clearly define and identify the different drug substances used in clinical trials in Spain, a guideline on nomenclature of cell-based medicinal products was followed [13]. This guideline, developed by AEMPS, defines not only the cell type but a number of additional attributes (tissue of origin, expansion in culture, other manipulations, etc.) as a pre-requisite to the final identification. The analysis of the products' characteristics showed in this paper took into account our register of PEI ATiMP.

Number and characteristics of the ATiMP in the authorized CT, owners of such products (commercial, i.e., pharmaceutical companies, or non-commercial, i.e., facilities within the National Health System) and number of CT per ATiMP have been analyzed.

The following aspects have been analyzed and verified for all authorised ATiMP CT during this period on the basis of information available on CT Applications and electronic CT Dossier Documents:

- Type of sponsor (commercial or non-commercial [14];
- Distribution of the CT according to type of ATiMP (sCTMP, GTMP, TEP) and GMO character;
- Phase of CT as indicated by the sponsor;
- Therapeutic area of investigation taking into account MeSH terms used by EudraCT [15] to define the Therapeutic Area;
- Population (i.e., adults (18–64 years), elderly (>65 years) and/or pediatrics (less than 18 years);
- National or International character taking into consideration geographical distribution of the participant sites;

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- According to the number of sites in Spain, single-site or multi-site CT;
- CT status and availability of results.

In addition, publication has been checked in REec [4], EU CTR [3] and ClinicalTrials.gov [16]. For CT found in these three registers, consistency in terms of the status displayed taking into consideration Table 2 and the availability of results with respect to the information received in AEMPS has been also reviewed. Taking into account that the status in REec reflects the situation of the CT in Spain, possible differences in the recruitment status among Registers for international CT have not been considered incoherent. Consistency of the end of trial status for international trials has also taken into account, if the global end of trial had been notified to the AEMPS. The latest available status from the following is shown, provided that at least one notification has been received for the trial in the last 2 years:

- *Not Initiated:* CT authorized, without reception of date of start;
- *Recruiting:* date of CT start received;
- *End of Recruitment:* date of end of recruitment has been provided;
- Temporarily Halted: temporary halt date has been received;
- *Prematurely Ended (According to the Regulation No 536/2014* [2], *early termination of a clinical trial means the premature end of a clinical trial due to any reason before the conditions specified in the protocol are complied with)/ Completed*: end of trial date has been received. CT having included a significantly lower than planned number of subjects or those not having completed all parts defined in the protocol have also been considered as prematurely ended for this analysis, even if the end was not notified as premature;
- *Unknown*: in cases where there have not been notifications by the sponsor within the last 2 years.

Results have been considered as:

- Yes: available results
- No: no available results
- NA (not applicable): when the CT has not finished yet or when

TABLE 2

Equivalence of CT status among the different CT Registries checked on this research.

REec	EUCTR	Clinicaltrials.gov
–	–	Unknown
Not initiated		Not yet recruiting
Recruiting (or restarted)	Ongoing (or restarted)	Recruiting
		Enrolling by invitation
End of recruitment		Active, not recruiting
Temporarily halted	Temporarily halted	Suspended
Prematurely ended	Prematurely ended	Terminated
		Withdrawn (no patients)
Completed	Completed	Completed

the global end date of the CT has taken place within the last year and the deadline to submit official results has not been reached yet, according to National Law and European Regulation

Verification of all CT status and results has taken place during September 2019.

RESULTS

During the period from 1st May 2004 to 30th June 2019, AEMPS received 331 valid CT applications on ATiMP that represent 2.9% of the total number of valid CT applications in that period. Status for these CT applications on 20th July 2019 (data analysis starting date) was: 290 authorized, 14 rejected, 19 withdrawn and 8 under assessment. Spain takes part in approximately 23% of ATiMP CT authorized in Europe [3].

CT according to type of ATiMP, sponsor & international character

The **distribution of authorized CT according to the type of ATiMP and sponsor** along the analyzed period is shown in Figure 1. Total numbers of ATiMP CT and distribution according to type of product, sponsor, country of the sponsor, international character, number of sites in Spain and phase is shown in Table 3.

Clinical investigation of ATiMP shows an important increase since 2010. Until 2013, it was mostly focused on sCTMP and TEP and driven by Spanish non-commercial sponsors (in fact, from 2005 to 2010 ATiMP CT sponsors are only Spanish). From 2011 onwards, sponsors from other countries started to sponsor ATiMP CT in Spain. The proportion of international sponsors increased up until 2018, when approximately half of the sponsors were from other countries (see Figure 2). In this sense, the figures from 2018 are especially revealing, since 29 out of the CT run by a sponsor not based in Spain were authorized. CT on GTMP are mainly run by commercial sponsors and have a greater relevance since 2016, showing a great peak in 2018 coinciding with international CT increase, as shown in Table 3. This is consistent with the evolution in the type of ATMP being investigated (see Figure 1).

Most ATiMP CT are early phases: Phase 1, Phase 1/2 and Phase 2 represent 80.3% of all authorized CT during the study period. Non-commercial sponsors are more focused on early phases clinical trials, as opposed to commercial sponsors who conduct the majority of Phase 2/3, 3 and 4 trials. There is no significant relationship between the type of therapy and phases of CT. Most national clinical trials have non-commercial sponsors (88%). International trials are mostly Phase 2 or 3, while national trials are Phase 1 and 2. Non-commercial sponsors mostly conduct single-site trials while multi-site trials are conducted by commercial sponsor (see Table 4).

Regarding the **Voluntary Harmonisation Procedure (VHP)**, available for CT planned to be conducted in two or more EU Member States, Spain has participated in the evaluation of seven CT with ATiMP by this procedure, five of

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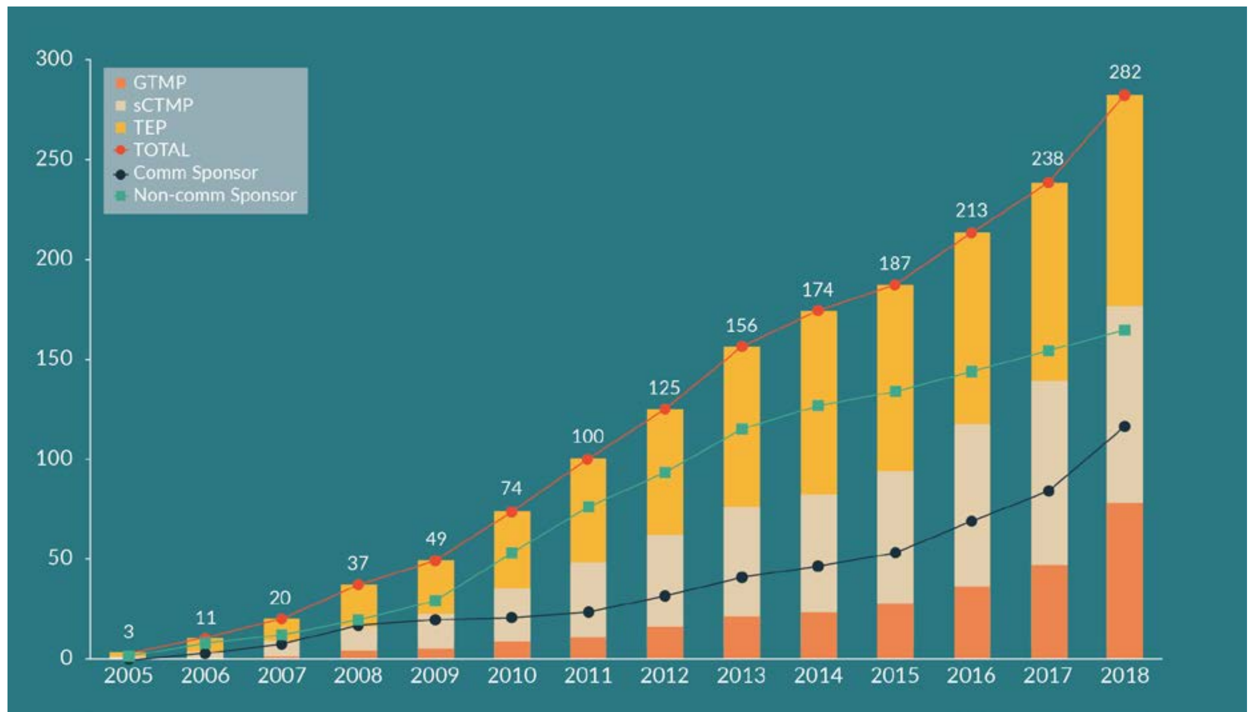
FIGURE 1

Cumulative data on authorized ATiMP CT in Spain (2005–2018).



which were with tissue engineered products. Most of these CT were Phase 2 or Phase 3, and both commercial and non-commercial sponsors used this procedure. Prevalence of these phases is also observed in all CT evaluated by VHP to date [6,7].

CT according to targeted disease & CT population

Globally, the most predominant therapeutic area was cancer (31.7%) followed by cardiovascular (14.8%) and musculoskeletal (10.0%) diseases. 63% of cancer CT investigated GTMP, while 35.9% of them investigated sCTMP. However, 95.5% of CT on the cardiovascular area and 96.4% of those on musculoskeletal diseases investigated TEP. The indications of leukemia/lymphoma/myeloma with 32 CTs, inflammatory bowel disease with 15 CTs, gastrointestinal system cancer with 13 CTs, and heart failure, ischemic and non-ischemic/cardiomyopathy with 13 CTs were the most frequent (Table 5).

17.9% of ATiMP CT include pediatric population (together with adults and/or elderly people [10.7%]; exclusively pediatric population [7.2%]). Most of these trials investigated GTMPs (48.1%) and have a commercial sponsor (61.5%). Regarding indication on exclusively pediatric CT, cancer remained the most prevalent (47.6%) followed by congenital, hereditary, and neonatal diseases and abnormalities (e.g., spinal muscular atrophy, Fanconi anemia, osteogenesis imperfecta, inborn errors of urea cycle, etc.; 38.1%).

CT were equally performed in both women and men.

TABLE 3

Number of CT on ATiMP according to type of product, sponsor, country of the sponsor, international character, number of sites in Spain and phase.

	No. OF CT ON ATiMP (N = 290)	No. OF CT ON sCTMP (N = 99)	No. OF CT ON TEP (N = 107)	No. OF CT ON GTMP (N = 84)
Sponsor				
Commercial	124 (42.8%)	32(32.3%)	21 (19.6%)	71 (84.5%)
Non-commercial	166 (57.2%)	67(67.7%)	86 (80.4%)	13 (15.5%)
Sponsor country				
Spain	209 (72.1%)	80 (81.0%)	94 (87.8%)	35 (41.7%)
USA	49 (16.9%)	9 (9.0%)	2 (1.9%)	38 (45.2%)
Rest of EU	29 (10.0%)	7(7.0%)	11 (10.3%)	11 (13.1%)
Israel	3 (1.0%)	3(3.0%)	0 (0%)	0 (0%)
International				
Yes	109 (37.6%)	27 (27.3%)	22 (20.6%)	60 (71.4%)
No	181 (62.4%)	72 (72.7%)	85 (79.4%)	24 (18.6%)
Sites in Spain				
Multi-site	156 (54.1%)	52 (52.5%)	49 (45.8%)	55 (65.5%)
Single-site	133 (45.9%)	46 (46.5%)	58 (54.2%)	29 (34.5%)
Phase				
Phase 1	71 (24.5%)	19 (19.2%)	28 (26.2%)	24 (28.6%)
Phase 1/2	33 (11.4%)	14 (14.1%)	1 (0.9%)	18 (21.4%)
Phase 2	128 (44.2%)	46 (46.5%)	61 (57.0%)	21 (25.0%)
Phase 2/3	3 (1.0%)	1 (1.0%)	1 (0.9%)	1 (1.2%)
Phase 3	52 (17.9%)	19 (19.2%)	14 (13.1%)	19 (22.6%)
Phase 4	3 (1.0%)	0 (0%)	2 (1.9%)	1 (1.2%)

ATiMP

Regarding **investigated products**, 168 different ATiMP products are being investigated in the authorized CT, being 54 (32.1%) TEP, 51 (30.3%) sTCMP and 49 (29.2%) GTMP, while 14 (8.3%) products are being investigated as both sTCMP and TEP. They include ATiMP currently having a marketing authorization in the EU (Alofisel®, Holoclar®, Imlygic®, Kymriah®, Yescarta® and Zalmoxis®), USA (Zolgensma®) or Spain (NC1). NC1 is a product prepared on a non-routine basis according to specific quality standards, and used within Spain in a hospital under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient, authorized by the AEMPS, as defined in Regulation

TABLE 4

National or International character and number of sites in Spain for CT on ATiMP according to type of sponsor.

	Non-commercial	Commercial
National (N = 181)	159 (87.8%)	22 (12.2%)
Single-site	103	5
Multi-site	56	17
International (N = 109)	7 (6.4%)	102 (93.6%)
Single-site	2	23
Multi-site	5	79

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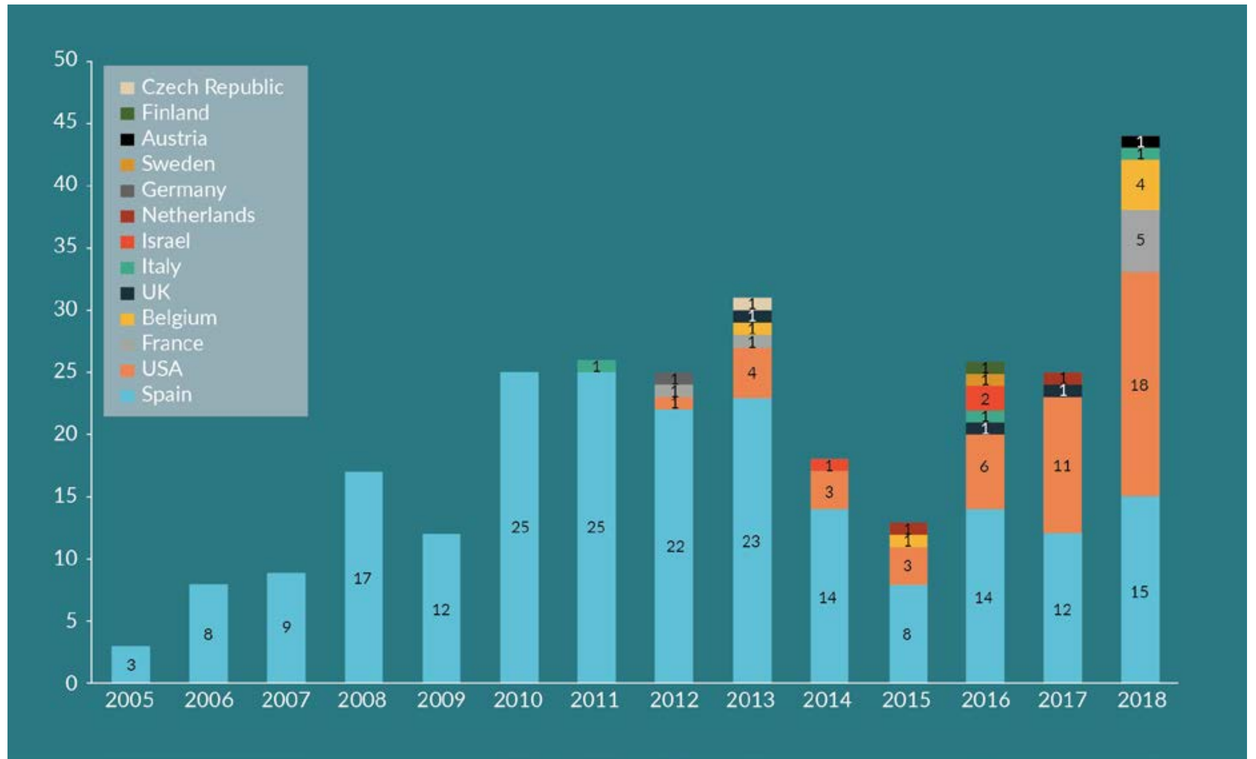
FIGURE 2

Authorized ATiMP CT per Sponsor country.



[EC] No 1394/2007 [8] and Royal Decree 477/2014 [17]).

40 of the GTMP and two of the sCTMP are GMO and they are being investigated in 75 CT. Their Product Owners are mainly commercial (85.7%). Sponsors for GMO CT are from Spain and USA (43% each), while sponsors for the other 14% are from other European countries.

The number of CT per PEI has ranged from 1 (for 112 ATiMP) to 9 (for 2 ATiMP). 21 products have been investigated on at least four CT, including Alofisel®, Imlygic® and Kymriah®, which have a marketing authorization in the EU, and NC1 (authorized in Spain according to the national legislation for 'hospital exemption'). Twelve out of these 21 products are manufactured in a facility pertaining to the national health system, while the other nine pertain to a pharmaceutical company.

It is remarkable that 91 out of 168 ATiMP belong to non-commercial owners; most of them are sTCMP and TEP, in consistency with the type of CT run by non-commercial sponsors. On the other hand, most of the products that belong to commercial owners are GTMP.

CT status

According to EU legislation, sponsors have the obligation to report National Competent Authorities relevant dates and information for the CT in order to make its status transparent. Certain information, such as the annual safety report, should be provided yearly along the CT duration. In addition, the

TABLE 5

Number of trials in the four most investigated disease areas: cancer, cardiovascular diseases, musculo-skeletal diseases and digestive system diseases.

Disease area	Diseases	Number of trials
Cancer	Leukemia/lymphoma/myeloma	32 (34.8%)
	Gastrointestinal system cancer	13 (14.1%)
	Brain cancer	9 (9.8%)
	Skin cancer	8 (8.7%)
	Bladder or renal cancer	5 (5.4%)
	Respiratory system cancer	4 (4.3%)
	Prostate cancer	3 (3.3%)
	Breast cancer	3 (3.3%)
	Others	15 (16.3%)
	TOTAL	92
Cardiovascular diseases	Heart failure, ischemic and non-ischemic/ cardiomyopathy	13 (30.2%)
	Myocardial infarction/coronary	12 (27.9%)
	Limb ischemia and peripheral arterial disease	12 (27.9%)
	Stroke	6 (14.0%)
	TOTAL	43
Musculoskeletal diseases	Joint or bone arthrosis	11 (37.9%)
	Bone defects	9 (31.1%)
	Spinal defects or pathology	6 (20.7%)
	Tendinopathy/ligament defects	3 (10.3%)
	TOTAL	29
Digestive system diseases	Inflammatory bowel diseases (perianal fistules)	15 (65.3%)
	Hepatic failure/cirrhosis	7 (30.4%)
	Fecal incontinence	1 (4.3%)
	TOTAL	23

CT should be published in the EU CTR [3] and in the REec [4] as is indicated in Table 1. However, considering the international character of part of the CT and the relevance of ClinicalTrials.gov [16] also for EU investigators and sponsors, the registration status of all ATiMP CT authorized in Spain has also been checked in that register.

All authorized ATiMP CT since 1st January 2013 (n=165) are registered and published in REec [4]. In 230 out of 290 CT, Spain is a participating country in a record in the EU CTR [3]. The 60 not published CT are phase I and do not include pediatric population. 235 CT are registered in ClinicalTrials.gov [16]. Only 8 CT, authorized before August 2011, are not published in any of these registers, and only 117 out of 290 CT are published in all of them.

Considering the information available at AEMPS, the status of ATiMP CT is reflected in Table 6.

The public status displayed for the CT published in REec, EU CTR [3] and ClinicalTrials.gov [16] was consistent in most cases (98 out of 117 cases). For 12 CT the status in ClinicalTrials.gov [16] was not updated according to the information available in the AEMPS and in 6 cases the information seemed to be more updated in ClinicalTrials.gov [16] than in the AEMPS. Coherence of the CT status in REec [4] and the status for Spain in EU CTR [3] is seen but this is expected since

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AEMPS is responsible for keeping it updated.

During the analyzed period, 142 CT were ended by the sponsor, 47 of which were terminated earlier than expected. Table 7 shows the number of prematurely ended clinical trials in relation to the reasons for it. The main reasons for early termination included lack of recruitment (53.2%) and business reasons (21.3%). It is remarkable that lack of efficacy and safety were the reason for the early termination in just five and two CT, respectively.

Regarding the time elapsed from the authorization until the early termination, less than 1 year passed in 31.9%, between 1 and 3 years in 27.6% and more than 3 years in 40.4%. Finally, the predominant therapeutic areas on these CT were cancer (40%), and cardiovascular diseases, coinciding with the two most investigated therapeutic areas for ATiMP CT.

ATiMP CT results

According to the EU legislation, sponsors should upload a summary of CT results in EudraCT [15]. These results are published in the EU CTR except if the CT is only Phase 1 and does not include pediatric population. Results should also be submitted to EU Member States National Competent Authorities. In both cases, the deadline for this submission is within a year of the end of trial (usually the last visit of the last patient). For CT authorized in Spain since 1st January 2013, the summary of the results is published at REec, except for Phase 1 CT not including pediatric population, for which there is limited information published.

According to these criteria, AEMPS should have received the summary of results for 73 completed CT for which the due date has expired. Twenty-three of these trials have a commercial sponsor while 50 of them have a non-commercial one. Results for only 45 CT (61.6%) have been received and only 14 CT have results publicly available either in REec (n= 4) [4], in EU CTR (n=7 plus intermediate results for 1 CT, authorized since 2007) [3] and/or in ClinicalTrials.gov (n=5 CT authorized since April 2012) [16].

With respect to prematurely ended CT, results are expected to

TABLE 6

Status of ATiMP CT according to information available in AEMPS.

CT status	Number of CT per status according to information available in AEMPS
Not initiated	16
Recruiting	74
End of recruitment	30
Temporary halted	3
Prematurely ended	46
Completed	79
Unknown	42

Unknown status for 17 CT authorized before 2013 might be due to the fact that for these trials part of the information could be in a paper File on CT, not checked for this review, which was previous to the current AEMPS database that contains all documents in the CT dossier presented in an electronic format.

TABLE 7

Number of prematurely ended clinical trials distributed by reasons for early termination (CT authorized before and after 2013).

Reasons	No. of CT (auth. < 2013)	No. of CT (auth. ≥ 2013)
Lack of recruitment	20	5
Business reasons	4	6
Enough data	1	1
Lack of efficacy	2	3
Safety	0	2
Other reasons	3	0
TOTAL	30	17

be made public as soon as possible within the year following the end of the CT, especially information related to safety or lack of efficacy, unless the CT ends with no relevant subject participation. Following these criteria, results have been accessible for the AEMPS in most of the cases (72.8%). Results are published in a CT register in seven cases, including all CT stopped due to lack of efficacy.

DISCUSSION

This article analyses all CT on ATiMP authorized by AEMPS from 2004 to 2019. This group of 290 studies represents around 22% of the entire CT with ATiMP conducted in the EU. As already stated, Spain is one of the countries in the world with more significant activity in this area [6,11].

The interest in identifying specific numbers for IMP investigated is highlighted, since these numbers are difficult to find due to the natural evolution of the names in products under clinical development. However, in the field of cell and tissue research, where the nature and origin of the cells as well as the autologous or allogeneic character could greatly influence the efficacy and safety of the products, having a more systematic way of describing the ATiMP under development could be of great interest as AEMPS has previously highlighted [13].

Regulation 1394/2007 [8] set a clear and common framework for ATMPs in the EU. This regulation, amongst other things, added tissue engineered products as a new class of ATMPs to the previously defined gene therapy and somatic cell therapy medicinal products. This meant that many cells and/or tissue-based treatments that were already in clinical use outside the pharmaceutical legislation, became regulated as medicinal products when the ATMP regulation came into force (December 2008).

Publication of the ATMP regulation clearly had a positive effect on the number of clinical trials in Spain, as observed by the increase from 2010 in Figure 1. At that time, most of the trials had an academic sponsor and research was mainly focused on TEP and sCTMP (Figure 1). The number of trials stayed relatively high up to 2014, when a clear drop is observed, presumably due to the restrictions in public investments in clinical research associated with the worst years of the economic crisis. Recovery in number of clinical trials started from 2016, but this time driven mainly by commercial research (Figure 1).

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The huge increase in the last 2 years is a clear reflection of the success in gene therapy clinical research directly related to industry (including big pharma) becoming increasingly interested in the development of advanced therapies (Figure 1) and expressed in the availability of several GTMP marketed both in the EU and USA since 2015 [18–20]. This increase has occurred despite the additional difficulties imposed on most gene therapy medicinal products because of their consideration as Genetically Modified Organisms (GMO). Application of the GMO regulation [21] in the EU to clinical trials with most gene therapy products means the involvement of a different competent authority to assess the potential environmental effects of such products, complicating the authorization procedure. This has a greater impact on multinational trials, because each MS has its own GMO competent authority and the procedures are far from harmonized across the EU. In an effort to unify criteria and streamline the process several activities have been initiated, which have already yielded a number of consensus documents [10]. Although these documents are not obligatory, a good number of MS (including Spain) have adopted them on a voluntary basis. This is expected to ease the administrative burden for clinical trials authorizations of medicinal products containing or consisting of GMOs.

The main characteristics of academic studies (n=166), as can be seen in Tables 3 & 4, are: early Phase 1, 1/2 and 2 CT (94%), national (95.8%), unicentric (62%) and focused on the investigation of sCTMP or TEP (92.2%). This is consistent with the logistical difficulties in organizing late-phase CT that normally involve hundred or even thousands of patients, and require the involvement of many investigators and sites in different countries. An example of these difficulties is highlighted in the article by investigators of the study MESEMS [22] which due to financial constrains has been designed to merge partially independent clinical trials. In fact, 91 out of 168 ATiMP investigated are produced within the National Health System in non-commercial GMP-compliant facilities. It is remarkable that sometimes the results of early academic studies are the basis for the further development of a marketed product as was the case for Alofisel [23]. The fact that only 6 of the non-commercial products were GTMP could be due to the more complex manufacturing process of these products.

On the other hand, 82.8% of the CT on Phase 2/3, 3 and 4 and 84.5% of the CT on GTMP are run by commercial sponsors. Additionally, commercial CT stand out in their international (82.3%) and multicenter characteristics, as can be seen in Tables 3 & 4. This is in line with the characteristics necessary for confirmatory CT required to support the application for the marketing authorization of any medicinal product.

Our results show that 290 ATiMPs CT were conducted in different therapeutic areas. Cancer, with almost a third of the trials (31.7%), cardiovascular (14.8%) and musculoskeletal (10.0%) diseases were the most prevalent ones. Cancer diseases were also prevalent for pediatric patients (47.6%) due to their severity and scarce therapeutic alternatives, as well as congenital diseases (38.1%).

Our results regarding indications are in line with the search in the main international CT databases performed by Hanna *et al.* [24] or the review of ATiMP CT between 2004 and 2010 by Maciulatitis *et al.* [11] in the EU, or even in European-country publications such as by the Czech Republic [25]. Although there were multiple indications, it is important to highlight refractory and recurrent characteristics, and the scarce and poor therapeutic alternatives for them (e.g., refractory and metastatic tumours, critical limb ischemia, non-revascularisable myocardium, complex perianal fistulas, osteogenesis imperfecta, spinal muscular atrophy, etc.). It is remarkable that 30.8% (n=16) of pediatric ATiMP CT were authorized in 2018. Indeed, 36.4% of authorized ATiMP CT in 2018 included pediatric population, while only 14.9% of total authorized CT (on any kind of medicinal product) in 2018 included pediatric population. This seems consistent with the increase in the GTMP CT.

Regarding the reasons that motivated a premature end for a CT, shown in Table 7, the main reason was lack of recruitment. The importance of a correct design that takes into account all actual population characteristics should be pointed out, in order to avoid lack of recruitment after all the efforts deployed to set up the trial. It is important to indicate that 30 of the prematurely ended CT were authorized before 2013, and for 20 of them, reasons for stopping the CT were related to a lack of feasibility in recruiting the necessary patients. This seems to indicate that nowadays, protocols are better adapted to true patient characteristics, which marks an improvement in their quality.

Only two CT on a GTMP have been stopped due to safety reasons, one of them with no patient participation because the safety problem was detected prior to enrolment. The time elapsed between the date of authorization of the CT and the premature end seems to be related to the reason for stopping the trial: the CT ended because of a lack of recruitment tended to last longer while those CT ended due to business or safety reasons usually stopped within the first year.

Currently, all CT should be uploaded to the European CT database EudraCT and be published in the EU CTR, except for those Phase 1 studies not including pediatric population. It is remarkable that the commercial confidentiality principle that supported hiding these Phase 1 CT not in the EU CTR for many years is not applicable in CT.gov, where 48 out of 60 non-pediatric Phase 1 CT are published.

Under-reporting of CT results is a serious problem which has been frequently highlighted [26–28]. Publication of results is not only an ethical issue but a legal requirement [1,2,14]. Our analysis shows that AEMPS has received an on-time report on the results for 65 out of 105 expected. 36 of these were with a commercial sponsor and 69 from a non-commercial sponsor. This means a rate of proper reporting of 72.2% for commercial and 53.6% for non-commercial sponsors, confirming the lower rate of reporting results for academic sponsors previously shown [29].

When looking into the structured format required to provide the results for the EU CTR [3] and ClinicalTrials.gov [16] registers, it is remarkable the fact that only 15 CT from

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commercial sponsors have results uploaded to EU CTR (out of 103 registered CT for which such results could be expected). In addition, only nine CT (five from non-commercial sponsors and four from commercial sponsor) out of 104 registered and for which results could be expected have results uploaded to ClinicalTrials.gov [16] with a similar structured format. However, for 27 CT (12 from academic sponsors and 13 from commercial ones) not having loaded the structured results as required, there is at least one paper in a medical journal focused on the results referenced within the record of the CT in ClinicalTrials.gov [16] as 'Publications automatically indexed to this study by ClinicalTrials.gov [16] Identifier (NCT Number)'. The lapsed time between the end of CT date and the publication has been 1 year for only one CT, longer than 1 and less than 2 years for nine CT, longer than 2 and up to 3 years for five CT, and longer than 3 years for the other ten CT.

These data could point to difficulties in in completing the current structured summary of results, especially for academic sponsors. In addition, this shows the need to increase awareness around the legal need for sponsors to organize CT activity in such a way that a summary of results could be available within 1 year of the end of CT date (usually the date of last visit of the last patient). It would be important that editors of Medical Journals do not reject the publication of CT results due to the public availability of the aforementioned legally required summary in official CT registers.

As this sample includes a big portion of non-commercial trials, this concern may not only be specific to ATiMP research but can also reflect the general difficulties related to academic research of medicinal products. It is remarkable that the vast majority of CT for which the status is unknown are old and non-commercial CT, showing that there has been an increasing interest in complying with regulations thanks to efforts from several networks such as STARS Project (Strengthening Training of Academia in Regulatory Science) [30] – an initiative funded by the EU with the aim of analyzing and improving training of non-commercial sponsors on regulatory science in order to have better and faster access to innovative therapies. In the case of Spain, AEMPS has created the Office for the Support of Innovation and Knowledge with Medicinal Products [31], responsible for giving technical and administrative advice to every innovative project that is going to take place in Spain or EU. Within this Office we can find a specific Office for non-commercial research, where special support from the beginning of projects is usually needed.

Regulation 536/2014 [2] is still not fully applicable in Europe. In the meantime, all stakeholders should get prepared to work according to the new CT Regulation rules. This implies (among other things) having a single national contact in the EU to organize access for the sponsor's users to the future EU CT database and portal on the basis of the *who does what* principle (viewer, preparer or submitter roles) and taking into account the future transparency rules [5] and the principle of having single consolidated documents for all MS [32] when preparing the CT dossier.

Transparency should be seen as an opportunity to identify serious health problems not yet investigated, to identify known risks to be avoided/minimized in future CT, to

facilitate recruitment and to cooperate with other sponsors (as well as many other positive things). However, to see this benefit, all stakeholders should commit to comply with this principle that is very much emphasized in the new EU legislation.

In the EU, the EudraCT number is a unique identifier necessary for all CT on medicinal products. It would be very helpful if medical Journals always required the inclusion of the EudraCT number, together with any other relevant identifier, in any publications related to this type of CT with participation of EU sites. Currently, this number is only present in about 56% of the CT records identified in ClinicalTrials.gov [16], but it would be very helpful if sponsors could reference this number every time the CT is identified for a CT Register.

VHP [7] has been the basis for the coordinated evaluation procedure established in EU Regulation 536/2014 [2]. For this reason, it is the perfect place for active adaptation to the changes that will be implemented by the new European Clinical Trials Regulation. By using VHP, sponsors will not only get experience on the European coordinated assessment but could also influence possible improvements on the application of the future legislation itself with real cases that are presented to us on a day-to-day basis. However, the VHP was only used by a minority of the international CT with ATiMP conducted in Spain. This may reflect a perception of a higher complexity for this procedure, especially by academic researches. In the case of GTMP, application of the GMO regulation may have also interfered with a harmonized assessment process between different MS, as described above. These issues will be taken into account for the implementation of the new Regulation.

Problems highlighted here especially for non-commercial trials may not be specific to ATiMP research, but can also reflect the general difficulties related to academic research on medicinal products due to the large number of this type of sponsor represented in this sample.

In Europe, there are several initiatives ongoing trying to facilitate CT under the scope of the new regulation. Discussions on possible improvement of VHP, simplification in the Environmental assessment of GMOs, the 'Strengthening training of academia in regulatory sciences and supporting regulatory scientific advice' (STARS) project, and an update of the guidance related to the CT Regulation in volume 10 Eudralex [33] are among them.

CONCLUSION

Clinical research on ATMP has seen a clear increase, especially on GTMP, during the last few years. This increase has been in parallel with an improvement in the quality of CT, highlighted with the rising number of multi-site and international CT (also a consequence of the increasingly commercial sponsoring of CT).

Our analysis also shows some difficulties in complying with regulatory requirements, especially for non-commercial sponsors. In this regard, it is notable that there are several initiatives at a European and Spanish level, such as the STARS Project [30] and European Commission initiatives to unify GMO requirements [10] in the EU, or the Office for the

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Support of Innovation and Knowledge with Medicinal Products [31] in Spain, that are trying to facilitate clinical research.

Last but not least, it should be noted that Regulation 536/2014 [2] is intended to be an instrument for cooperation between EU MS and sponsors in order to ease the regulatory framework burden and promote clinical research in the EU. Since this regulation is not yet fully applicable, all stakeholders still have time to adapt their workflows and national legislations to the new way that lies ahead.

AUTHORSHIP & CONFLICT OF INTEREST

***Contributions:** All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR ADVANCED THERAPIES

SPOTLIGHT

INTERVIEW

Keys to success for foundation: industry clinical development collaborations

BRIAN FISKE joined The Michael J. Fox Foundation for Parkinson's Research (MJFF) in 2004. As Senior Vice President, Research Programs, Brian co-manages a team of professionals who stay closely linked to the Parkinson's community in order to develop an aggressive and innovative agenda for accelerating research and drug development for Parkinson's disease. This ensures that MJFF priorities reflect and best serve the ultimate needs of patients. Brian regularly meets with academic and industry scientists around the world to identify promising ideas to support, providing troubleshooting and ongoing management of projects as they go forward. He currently oversees the teams focused on MJFF's strategies for developing disease-modifying and symptomatic therapies for Parkinson's patients. Brian earned an undergraduate degree in biology from Texas A&M University and a PhD in Neuroscience from the University of Virginia. After completing postdoctoral research at Columbia University, Brian spent several years as an editor for the prestigious scientific journal, Nature Neuroscience. He brings this broad experience and knowledge to the Foundation to help bring new treatments to people with Parkinson's.

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Q Can you firstly give us some background on The Michael J. Fox Foundation for Parkinson's Research (MJFF)'s involvement to date in the clinical development of gene therapy product candidates?

BF: The Foundation has been around since the year 2000 and from day one we have been focused on accelerating and enabling therapeutic development for the more than one million people living with Parkinson's disease (PD) in the USA today, and the many more patients living with the disease globally.

I've personally been with the Foundation for 15 years and it's been amazing to watch the evolution of the therapeutic pipeline for PD over that time and how robust and compelling it has become. There are a lot of diverse approaches being tested for PD, including traditional pharmacological treatments but also innovative approaches using gene therapy as well as some cell-based therapies.

In the early years of the Foundation, some of the leading therapeutic approaches included brain tissue transplantation therapy and the delivery of certain types of growth factors and other proteins in the brain. One of our first experiences in helping with the clinical development of a gene therapy came around this time when we began to work with a company called Ceregene. They were developing a gene therapy approach for a growth factor called neurturin – their hope was to deliver this potentially protective growth factor into the brains of people with PD to help keep dopamine-producing cells alive. Unfortunately, the trials testing neurturin did not show benefits so the program was halted. But it represented some of our first experiences working with a gene therapy program.

Since that time, we've continued to support lots of different approaches. Not all have been gene therapies, but we have certainly seen that field continue to grow and evolve, and when you look at the pipeline today, there are at least a handful of gene therapy approaches in clinical development. A couple of these (Voyager Therapeutics/Neurocrine and Oxford BioMedica/Axovant) are trying to deliver some of the synthetic machinery for making more dopamine in the brain, while other companies are continuing down the path of delivering protective factors – one recent example is Prevail Therapeutics who have a gene therapy program for PD with GBA1 mutation (PD-GBA).

So we're certainly starting to see some interesting movement and exploration of gene therapy in PD.

Q What does the Foundation seek to bring to its clinical development collaborations in terms of capabilities and expertise?

BF: One of the ways we can provide support is financially. Since our early days, we've had mechanisms in place to provide grant funding for

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individuals and groups to help them develop novel therapeutics, and make the case for them being relevant and promising for PD.

However, over the years, we've developed a sophisticated approach that now includes multiple ways in which we can support drug developers as they move forward, beyond simply funding them. For example, we have teams here that are expert in understanding the challenges of recruiting patients into PD trials, and the different ways we can engage patient groups to educate them on the value of their participation.

"...the biggest 'do' when thinking about collaborating with an organization such as ours is to treat us as a partner."

The Foundation is at the nexus of R&D activity in PD, so we have connections to a lot of different external expertise that can also be leveraged. When a company approaches us with a therapeutic idea, they may be struggling to find a good PD expert who can participate on their scientific advisory board, or maybe they're looking for input directly from patients – they may want to hear from a certain patient group about the particular type of treatment they're developing. We have ways to engage such individuals and groups – to connect those dots and help companies access the expertise they need.

So we have really expanded the menu of opportunities to help support therapeutic development for PD.

Q Can you summarize the key learnings from your years of experience collaborating with the gene therapy industry and academia in coordinating clinical development projects?

BF: It's been interesting over the years to figure out the best way to work with companies, in particular. It's one thing to work with academic groups that are more used to the idea of a foundation or a funder helping support R&D, but when you're dealing with companies, it's a different ballgame. There are different incentives involved, and there are many more concerns and considerations relating to proprietary information, for example.

In general, we've found that the biggest 'do' when thinking about collaborating with an organization such as ours is to treat us as a partner. A company can come to us and be open and honest about what they're trying to do in PD – let us know why they think it's important, what challenges they're facing. Again, the assistance we can provide is not limited to the purely financial. There are many different ways we can assist a company

that's developing a treatment for PD, but we can only help if we know what those challenges are – it's vitally important to have open and honest communication to make a collaboration work well.

> "...over the last 20 years or so, there's been a growing appreciation that Parkinson's disease isn't just about loss of one certain subset of brain cells..."

We've found in a number of cases that a particular problem raised by one company is in fact shared by multiple other companies in the same therapeutic space. There are often opportunities in these instances to look at pre-competitive initiatives as a means of solving the problems, and the Foundation can act in many ways as a neutral convener. We can help both to identify potential solutions and then ultimately, if everyone agrees it's valuable, actually take the lead role in supporting a particular project or study to address that challenge. It's obviously useful for every company involved to be a part of such collaborations because they all benefit from the outcome.

We do find that companies that are able to appreciate the value of that kind of pre-competitive communication and collaboration are the ones that often benefit the most from the work we can do, and the type of partnership we can build with them. We have lots of examples where this type of multiple stakeholder collaboration has worked, both in the context of broad programs – for example, general biomarker development in PD– as well as some very specific therapeutic challenges that have arisen, where the Foundation was able to step in and clarify through funded studies the issues that were causing concern to the benefit of all involved.

Q What are some of the key specific challenges involved in designing and conducting clinical trials for gene therapies against PD?

BF: Probably the biggest challenge is delivery, especially if the presumed mechanism of action through which the therapeutic gene is supposed to work is in the brain. So when you're thinking about designing trials for a gene therapy product in PD, at least with technology today, that right there is your first big hurdle: you're most likely going to be doing brain surgery in individuals with the disease. You need to ask what are some of the implications that come with that fact: how are you going to deliver the product? Is it a validated delivery approach and device? What are the other considerations for someone with PD who has movement and other potential disabling symptoms – how could that impact the surgical procedures you might put in place? PD is also generally a disease

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of older age, which is another thing to factor in when considering recovery from something as significant as brain surgery.

I think the other challenge is that over the last 20 years or so, there's been a growing appreciation that PD isn't just about loss of one certain subset of brain cells – in this case, the idea that it's all about loss of certain dopamine producing cells in the brain – but that it's really more of a 'whole body' disease. There are a lot of parts of the brain, even of the peripheral nervous system, that might be impacted by the disease. The idea of targeting a gene therapy product to just one region of the brain may help restore some function or protect cells in that specific region, but there's the whole rest of the body to consider, and the rest of the disease process that might still be happening around it. I think that with gene therapy, you have to appreciate this is a reality with PD: when you develop these types of treatment you have to understand that wherever you target it, you might only be addressing one component and not the entire disease process.

This is all based upon gene therapy in its current form and existing targeted delivery methodologies, of course. If over time we are able to advance gene therapy to a point where you can truly deliver it in the same way you might deliver a small molecule – systemically to the whole body – and if we know we can target the right cells and produce the appropriate therapeutic response, then that equation might change. You might then see gene therapy becoming more of a 'full disease' type of approach for PD.

A third component would be deciding what gene you want to deliver. Looking at the current gene therapy development pipeline, two of the current leading groups mentioned above are essentially delivering the enzymes for making more dopamine in the brain, which is certainly a valid approach and an interesting way of targeting the disease given loss of dopamine underlies much of the movement challenges seen in PD. You also have groups trying to deliver genes that target specific mechanisms believed to underlie disease cause – approaches which could potentially be restorative. But again, if these approaches only target a specific region of the brain, it may not necessarily affect the entire body. So it's important to be clear about what you think your gene therapy product is doing and where.

Q Are there any particular emerging cell and gene therapy approaches on your radar which may hold promise?

BF: We're certainly aware of and excited by the development of various approaches in the wider cell and gene therapy space and the potential opportunities they might bring to PD – we're keeping a close watch on them. We've seen genetically engineered cell

therapies have such a strong impact in oncology, for example – it will be interesting to see if certain transplanted cell populations can be genetically modified to provide factors that could be protective in PD.

Obviously, gene therapy in itself is not a treatment – it's really a platform technology for delivering potentially therapeutic genes. We tend to look at it that way: we don't see 'gene therapy' as the cure for PD, because it really depends on what it is you're delivering. But it's always exciting for us to see different novel platforms emerging. For me, the real promise for something like cell and gene therapy is that it is a comparatively very targeted, exquisite way of addressing a specific mechanism. It's not a traditional small molecule that might hit a bunch of different biologies – it's not always easy to chemically dial those off-target effects out. The enormous value that cell and gene can bring to developing treatments for a disease like PD certainly isn't lost on us – it's why we continue to monitor the field to see where the next opportunity may arise.

> "It's core to our mission that we constantly seek to push and accelerate the pipeline for new treatments for Parkinson's disease..."

Q Moving further forward, what are The Michael J. Fox Foundation's chief R&D priorities and goals for the future?

BF: I'll come back to the fact that the current R&D pipeline is probably the most exciting, healthy and robust that it's been in years. Lots of different approaches being tested, some addressing disease mechanisms, some aimed at providing better ways to handle the most serious disabling symptoms, and others seeking to address different stages of the disease. So we see this nice mix in the clinical pipeline for PD that is giving a lot of us hope and excitement about the opportunities in the years ahead. (In addition, over the last couple of years we have seen a number of new products actually getting approved for PD, which is always very important to see).

It's core to our mission that we constantly seek to push and accelerate the pipeline for new treatments for PD – we'll continue to develop both our strategic funding and our non-funding mechanisms for how best to enable that progress. One challenge we're seeing in clinical trial patient recruitment is that increasingly, treatments in development for PD are looking to target genetic forms of the disease. It obviously further complicates the patient recruitment picture when you have to think about how to identify, screen, recruit and enroll people with certain mutations linked to PD. So

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we and others in the community continue to develop strategies around how do we find these people and educate them about the valuable role they could play in developing drugs for PD so they can actually participate in some of these trials.

There's also been a lot of conversation lately around the regulatory paths for developing drugs for diseases like PD, so we have a whole separate effort ongoing that focuses on how we engage with regulators – and with payers, too. How do we develop lines of communication with these critical stakeholders and bring that insight to bear in identifying a clearer path for developing drugs for PD moving forward?

In short, there's a whole algorithm here in terms of how we accelerate the pipeline, which is a combination of several different types of strategies we can put in place. That is what we will continue to push in the coming years.

AFFILIATION

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The Michael J. Fox Foundation for Parkinson's Research

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CLINICAL TRIAL DESIGNS FOR ADVANCED THERAPIES

SPOTLIGHT

INTERVIEW

Breaking new ground: bringing an iPS cell therapy to the clinic

KAPIL BHARTI holds a bachelor's degree in biophysics from the Panjab University in Chandigarh, India, where he graduated with highest honors. This was followed by a Masters degree in biotechnology at the Maharaja Sayaji Rao University in Baroda, India and a diploma in molecular cell biology at the Johann Wolfgang Goethe University at Frankfurt in Germany. Supported by an international PhD student fellowship, he obtained his PhD from the same institution, graduating summa cum laude. His PhD work involved basic biology in the areas of heat stress, cellular chaperones, and epigenetics. From Germany, Dr Bharti came to the National Institute of Neurological Disorders and Stroke to work with Dr Heinz Arnheiter as a postdoctoral fellow. While there, he published numerous papers in the areas of transcription factor regulation, pigment cell biology, and the developmental biology of the eye. It is perhaps this combination of diverse backgrounds that led him to develop an interest in the emerging field of stem cell biology, particularly of the retinal pigment epithelium, as he moved into the role of staff scientist. Dr Bharti has authored numerous publications and has won several awards, including, most recently, being a finalist in the prestigious trans-NIH Earl Stadtman Symposium.

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What are you working on right now?

KB: The main focus of my lab at the National Eye Institute at NIH is to better understand the mechanisms of various forms of

retinal degenerative diseases, and to try to develop new therapies for them.

All the work in my lab is based around the use of induced pluripotent stem cells (iPSC). One of the main focuses for us is to develop an autologous iPS cell-based therapy for a disease called age-related macular degeneration (AMD), which causes patients to go blind later in life. AMD is thought to be caused by degeneration of an eye tissue. We have actually developed this tissue in the lab from patient-derived iPS cells and we're now trying to develop a Phase 1 clinical trial for those patients.

Q How do you go about approaching clinical translation in a novel and ground-breaking technology field such as iPSCs? What are the key considerations/lessons that might be generally applicable for others in the cell and gene therapy area?

KB: For me, one of the key things in the lab is to not give up the science – to really focus on the biology and science of the cells. When we say we're trying to do translational research, that's not to say we're not doing basic science research, too. For me, the two go hand-in-hand. It's really fundamental to understand everything basic about your cells, your gene therapies, whatever technology you are working on, before you introduce them into clinical application. The more you understand, the more likely it is they will be safe and efficacious in patients.

To give you one example, regarding the eye tissue we're making from iPS cells, we spent years using developmental biology of various organisms that had been studied, including mice and humans, and using that developmental biology to make sure that the cells we are making in a dish are as close as possible to native eye cells. Simultaneously, we spent a lot of time characterizing them to really gain an understanding of how they function. I think all those fundamental discoveries are really key to ensuring that your translation is going to be fruitful and will go forward properly.

I think the same thing is applicable to any technology: not to give up the basic biology.

Q More specifically, what are/were the main challenges in bringing an iPSC-derived cell therapy to first in human trials and how have you sought to address them?

KB: There's not a lot of work that's been done to date on bringing iPS cells to patients – there's just one example from Japan,

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essentially. There's not much background information on how to do it and that means a lot of work in both ensuring the safety of the cell therapy product, and also ensuring that you're making it correctly. As we're moving towards the trial, we're having to invent all of those things and to make sure that we're doing it right – it's a very steep learning curve in that sense.

> "One of the main focuses for us is to develop an autologous iPS cell-based therapy for a disease called age-related macular degeneration..."

The field knows that a key concern with iPS cells is that any cells left in the final product can be tumorigenic – you need to ensure that the final product cells don't carry any oncogenic mutations. We spent quite some time on this aspect to ensure our final product does not carry any iPS cells. In fact, we demonstrated that even if you forced iPS cells into the final product, they won't grow under the conditions we used to grow our test article – the one we want to transplant into patients. Even so, we went to great lengths to demonstrate in preclinical animal models that the cells don't form tumors or teratomas or migrate into any other tissue.

As part of this work to ensure the safety of the cells, we developed a process of making iPS cells from patients' progenitor blood cells – CD34-positive cells – that helped ensure that the cells maintained their proliferation early on (so that they could make the iPS cells) but would not accumulate mutation during the culture process. And in fact, we then checked for the oncogene and found out that in most cases, we don't see any potential oncogenic mutations in these cells.

In terms of delivery, we then had to develop a tool that really fits the back of the eye, that is safe for delivery, and at that is biocompatible whilst also capable of helping maneuver the transplant.

With all of this work done, we are right now in the process of working with the FDA towards our clinical trial approval.

Q Tell us about the trial design you have chosen for the first in human study in AMD patients – what have you selected in this regard and why?

KB: Since this is a Phase 1 trial, it is a safety study by design – we have discussed our trial design with the data monitoring board and the Institutional Review Board (IRB) and the main goal is to ensure that the transplant will stay safe.

What that means for us is that at least in the first cohort, we're transplanting into patients who already have significant vision loss, meaning that if the transplant were to prove to be unsafe, it wouldn't cause further

vision loss in those patients. Essentially, what we're looking for in the first cohort is safety of implant and whether it integrates in the back of the eye.

As we continue and we keep demonstrating safety of the transplant in the first cohort, we might be able to test it in a second cohort of patients who have slightly better vision. But again, the primary outcome is going to be safety throughout the trial.

Q There has been some discussion around the optimal method of delivery used for cell therapies against AMD – can you summarize your observations and opinions in this regard?

KB: There are multiple ways to deliver retinal pigment epithelium cells into the back of the eye. One of the first approaches was injecting a suspension of cells, and then people have also tried monolayer patch on a plastic scaffold. We took a slightly different approach: we're trying to transplant monolayer patch on a biodegradable scaffold.

I've spent a lot of time studying how RPE cells function and it is clear that these cells need to be fully polarized before they can perform any functions. What I mean by 'fully polarized' is you need polarized tissue that contains several thousand cells – a single RPE cell won't be able to perform all the functions that normal, native RPE tissue performs.

Because of this, we knew that to get optimal efficacy we would need to be able to deliver the patch as a polarized tissue in the back of the eye. Cell suspension may work under some conditions, but in many cases the integration of those cells is extremely challenging. And it's a large assumption that one would have to make to say that all the cells injected would form a perfectly polarized monolayer in the back of the eye. There's no real data that supports that.

In our experiments we did see some integration of suspension injections, but not at all at a comparable level to the integration of the monolayer patch. That's why we think that if the integration is so dramatically different between the two approaches, the efficacy will be dramatically different, too. But again, there's not enough patient data at this point to say for certain that one approach is definitely better than the other – this is all based on preclinical work.

> "...what we're looking for in the first cohort is safety of implant and whether it integrates in the back of the eye.."

Plastic versus biodegradable: in our hands, both worked well. For me, though, biodegradable intuitively makes more sense. As we allow the cells to form a monolayer, they secrete their own exocellular matrix and then the

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scaffold, which is no longer needed, simply degrades away. The scaffold is only designed for proper delivery in the right place – after that it is no longer required, so why keep it there? But we'll find out in due course if our approach really enables the longer-term survival of the patch as opposed to the plastic scaffold.

> "We hope that we can transplant 12 patients in the Phase 1 trial over the course of the next 2 or 3 years."

Q Changing tack for a moment, how do you think the 'auto vs allo' debate will play out in the iPS cell field?

KB: When iPS cells were first discovered in 2007, everyone was excited that for the first time we had the possibility of developing autologous cell therapies, because at that time, everyone accepted that allogeneic cell therapies would not work in the long-term due to immune rejection.

But nobody did the actual experiment of comparing an autologous iPS cell therapy to an allogeneic one, and to this day, still nobody has done that experiment! So I went with the approach of let's at least prove that autologous iPS cell therapy does work in a Phase 1 trial, and hopefully beyond that. There will then be a way to compare autologous with allogeneic.

The appeal of allogeneic cell therapy is of course that the manufacturing process is that much simpler. You would only have to make one cell bank whereas currently we have to make cells for every single patient, which is a lot more time-consuming and challenging in terms of financial resources. Clearly, if allogeneic does work (by which I mean no immune rejection of alloantigens or allotransplants) then that's the way to go. But as of right now, we don't know for sure if allo will work – that's why at this stage, I think we should be comparing the two.

Moving forward, if allo turns out to work at least as well as auto in our application area, and if there's a way to switch, I think it would be perfect for us to do so. Perhaps this will be made possible by using universal donor cells, which is where you take an iPS or embryonic stem (ES) cell line and knock down or knock out the actual antigens so that they are not seen by the host's immune system. However, while that approach is very intriguing, it has its own challenges: if the immune system doesn't see the allo cells at all and those cells then make a tumor, the immune system won't see that either. So we'll see how far that goes. But again, if it works, that is definitely the most appealing idea.

An intermediate approach is making iPS cell banks from individuals homozygous for certain MHC-haplotypes. The thinking there is that these cell banks could be applicable to a relatively large percentage of the patient

population, especially in societies that are not particularly ethnically diverse. It wouldn't work so well in the USA, but in Japan, for instance, one estimate is you would only need around 100 banks to cover more than half of the total population.

But at this stage, we really don't know which approach will be successful in the end. And I think that in the long-term, if autologous transplants do integrate and become efficacious, healthcare or health insurance companies will figure out a way to pay for them. And the cost could potentially be reduced by automation, of course – we all know the challenges in manufacturing brought about by the amount of manual labor required, which increases cost and therefore price. We and many other groups are working hard on trying to figure that one out.

Q Finally, can you share your chief priorities and goals for the next 12–24 months?

KB: The main priority is to work towards getting the IND approval for the Phase 1 trial and then getting that started. We're very close – we hope if things go well, we might get it in the next several months.

Once that approval is achieved, we'll then work towards transplanting a few patients next year. We hope that we can transplant 12 patients in the Phase 1 trial over the course of the next 2 or 3 years. In the meantime, we need to work out if the Phase 1 is successful, where we want to go from there for the Phase 2. Do we want to work on automation, or a hybrid approach for an allo cell therapy? We are discussing a lot of these possibilities at this moment but at the end of the day, the early Phase 1 data will dictate the direction we subsequently take.

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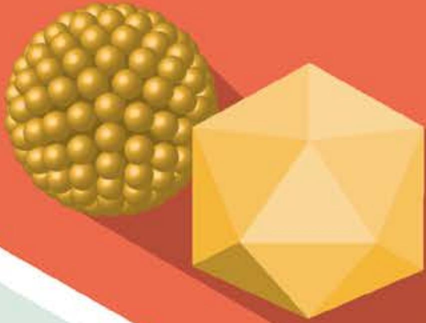
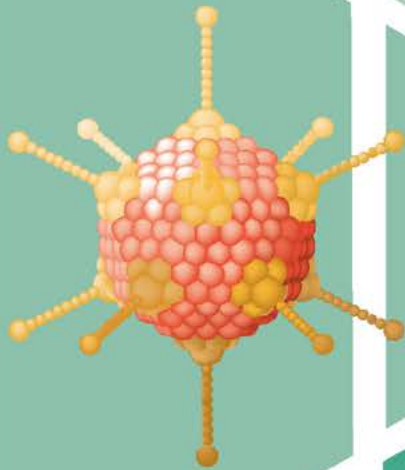
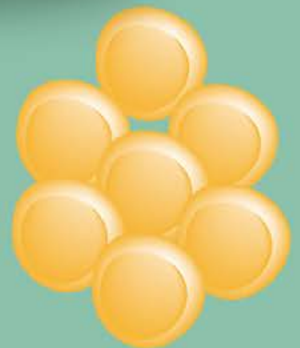
CELL & GENE THERAPY INSIGHTS

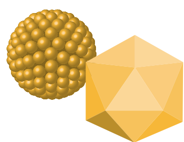
Viral Vector Channel



ADHERENT CULTURE METHODS EDITION







IN FOCUS:
Highlights from our Vector Channel

Adherent Culture Methods

EXPERT INSIGHT

Evaluation of AAV vector production from the iCELLis fixed bed bioreactor vessel
Shelley Nass, Bindu Nambiar, Maryellen Mattingly, Denise Woodcock & Catherine O'Riordan

1461–1471

INTERVIEW

Assessing the future prospects of upstream bioprocessing systems for commercial AAV production
Scott A Jeffers

1275–1279

CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: ADHERENT CULTURE METHODS

EXPERT INSIGHT

Evaluation of AAV vector production from the iCELLis fixed bed bioreactor vessel

Shelley Nass, Bindu Nambiar, Maryellen Mattingly, Denise Woodcock & Catherine O'Riordan

AAV gene therapy vectors have demonstrated efficacy in numerous clinical trials, and gene therapy products are now a reality. In support of the commercialization of AAV gene therapy biologics, scalable, high capacity AAV production methods are necessary, and here we describe the use of the iCELLis® Fixed Bed Bioreactor Vessel (Pall Corporation), a versatile AAV production system that can be used for the production of both research grade and GMP AAV vectors. The iCELLis® system is ideally suited for use with the triple transfection AAV production method utilizing adherent HEK 293 cells. For routine AAV research vector production in the iCELLis® Nano high compaction 4 m^2 vessel is used, with the option to combine up to four vessels in tandem, if vector yields greater than 1×10^{14} VGs are required. The use of the iCELLis® system provides a continuum in the vector production platform for pre-clinical AAV vector production to GMP AAV production for clinical trials and commercialization.

Cell & Gene Therapy Insights 2019; 5(11), 1461–1471

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MATERIAL & METHODS

AAV vectors were produced via transient transfection on planar vessels as previously described [1]. Briefly, HEK293 cells were transfected using polyethyleneimine, (PEI), and a 1:1:1 ratio of the three plasmids (inverted terminal repeat [ITR] vector, AAV rep/cap, and Ad helper

plasmid). The pAd helper used was pHelper (Stratagene/Agilent Technologies). Cell pellets were harvested following centrifugation (1,500 rpm for 15 min) and resuspended in lysis buffer (20 mM Tris [pH 7.5], 150 mM NaCl, 10 mM MgCl2) prior to freeze/thawing [1]. Following the addition of Benzonase® and 0.1%Triton™ X-100, the lysate was incubated at 37°C for 90 min and then centrifuged at 3,500 rpm before sequential filtrations using 0.8 µm and 0.45 µm filters. Purification of AAV was achieved using a column purification method as described previously [1].

SAMPLE PREPARATION FOR AUC ANALYSIS

The purified vector, at a concentration of 2×10^{12} to 5×10^{12} VGs/ml, was buffer exchanged into PBS (pH 7.2) using a 10K MWCO Slide-a-Lyzer™ (Thermo Scientific). The AAV vector absorbance signal was determined by optical density measurement at 260 nm (OD260) using spectrophotometric methods. For consistency, the samples were adjusted to a target concentration (OD260 of between 0.2 and 0.8) either by direct dilution with PBS or further concentrated using an Amicon® Ultra-0.5/30K MWCO Centrifugal Filter Device (Millipore).

SEDIMENTATION VELOCITY AUC DATA ACQUISITION

Sedimentation velocity AUC (SV-AUC) analysis was performed using a Proteome Lab™ XL-I (Beckman Coulter, Indianapolis, IN, USA). A 400 µl volume of sample was loaded into the sample sector of a two-sector velocity cell, and 410 µl of PBS was loaded into the corresponding reference sector. The sample was placed in the four-hole rotor and allowed to equilibrate in the instrument until a temperature of 20oC and full vacuum were maintained for 1 h. Sedimentation velocity centrifugation was performed at 20,000 rpm and 20oC. Absorbance (260 nm) optics was used to record the radial concentration as a function of time until the lightest sedimenting component had cleared the optical window (1.2 h). AUC data were analyzed as previously described [2].

QUANTITATIVE PCR ANALYSES

The AAV vector was quantified using a real-time qPCR assay (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA, USA) with primers specific for the polyadenylation signal. Vector levels are expressed as vector genomes per milliliter, VGs/ml.

ANALYZING RAAV VECTOR PURITY USING SYPRO® RUBY PROTEIN GEL STAIN

Samples from purified vector were loaded onto a NuPage™ 4-12 % Bis-Tris gel (Invitrogen). Typically, 5×10^{10} VGs of purified vector was analyzed. The gel was stained with SYPRO® Ruby Protein Gel Stain (Life Technologies) and observed under a UV light source.

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IN VITRO TRANSDUCTION ASSESSMENT OF AAV

HEK293 cells were seeded at 2×10^5 cells/well and infected 24h later, in triplicate, with AAV at a MOI of 1×10^6 VGs/cell in a 500 µl volume. The media were replaced 24 h post-infection with 1 ml of complete DMEM containing 10% fetal bovine serum (FBS), penicillin/streptomycin (pen/strep), and L-glutamine. After 72 h, cells were lysed and assayed for vector genome copy number by qPCR assay (BGH target) and eGFP protein levels using an eGFP ELISA® kit from Abcam (ab 171581).

OPERATION OF THE ICELLIS® FIXED BED BIOREACTOR

The iCELLis® Fixed Bed Bioreactor system is a disposable bioreactor vessel that is available with multiple surface growth areas **(Figure 1)**. The smaller scale unit, the iCELLis® Nano, ranges in cell growth surface area from 0.53–4.0 m², while a larger manufacturing unit, the iCELLis® 500+ provides up to 500 m² of cell growth surface area. Potential advantages to the iCELLis® Nano bioreactor include pH and temperature control along with the replenishment of O_2 during the AAV vector production process, promoting optimal cell viability. The iCELLis® bioreactor consists of a fixed bed surrounded by culture medium. The medium is pumped from the bottom of the bioreactor through the bed and then falls as a thin-film down the outer wall of the fixed-bed. The O_2 is depleted from the media but is replenished within the headspace above, as O_2 is fed into the bioreactor.

The iCELLis® Nano vessels were prepared according to the manufacturer's recommendations with the appropriate Pall branded consumable parts, DO (Dissolved Oxygen Probe used to measure oxygen content in the vessel during the duration of run), and pH probes. Following autoclaving, the vessels were filled with 700ml DMEM supplemented with 5% FBS and allowed to condition over night at 37°C, with stirring at a linear speed of 2 cm/s. Prior to inoculation with cells, the DO and pH probes were recalibrated, activated and allowed to stabilize for 60 minutes. The following set points were used throughout the duration of the run, DO: 45%, pH: 7.2 and temperature: 37°C. Air flow was also activated at this time, at a rate of 30 ml/min. Pre-cultured HEK293 cells (Agilent) were introduced to the Nano vessels in a concentrated volume of 100 ml for a total of 4×10^8 cells per 4 m² iCELLis® Nano vessel (10,000 cells/cm²). To maximize cell attachment and initial cell growth in the concentrated media environment, the speed of the stirrer was adjusted to maintain the 2 cm/s linear speed, while the circulation of medium was initiated 6–8 h post cell inoculation. Typically, 4 l of DMEM supplemented with 5% FBS was pumped into the iCELLis® Nano vessel at a rate of 24 ml/min, and subsequently cycled out of the vessel at a rate of 28 ml/min. The increased outlet circulation rate prevents the headspace from inadvertently becoming too small which could reduce overall gas exchange. The working vessel volume remained at approximately 668 ml throughout the duration of the perfusion event. A

FIGURE 1

The vessels are controlled by a stand-alone mPath bioreactor control tower and Pall Link; a supervisory control and data acquisition (SCADA) software package.

A center column within the vessel provides a fixed bed of polyester microfibers for cell attachment and expansion. The bottom left image shows a microscopic view of cell attachment to the microfibers. (Pall Corporation).

7 cm piece of tubing attached to the 'Media Out Port' on the underside of the Nano vessel lid, gave a 6 cm falling film height. The falling film height can be adjusted by altering the length of tubing allowing for increased gas exchange if necessary. The vessel will not inadvertently empty overtime because media is only removed when the media level rises to the level of the tubing. Cell growth proceeded for 72 h with daily sampling of media from the vessels using a ViCell MetaFlex™ system (Beckman Coulter). This provided an offline measurement of the levels of pH, DO, and media nutrients and metabolites. Cells were transfected using the triple transfection method, as described [1]. Prior to transfection, a cell count was performed by aseptically removing carriers from the Nano vessel and performing a cell nuclei count. The top GL45 cap of the vessel was removed in the hood and sterile tweezers were used to manually remove two carrier strips from different areas of the fixed bed. The cell strips were placed in a snap top tube containing 300 µl of PBS. The cells were then lysed from the strips, by adding 300 µl of Lysis Buffer Reagent A100 as described by the vendor (Chemoetec) and vortexing for 1–2 min. An additional 300 µl of Reagent B was then added and vortexed to stabilize the sample. A nuclei count was performed on a sample loaded into a Via1-Cassette on the NucleoCounter® NC-200 System (Chemometec); since the strips have a known surface area of 13.9 cm^2 a cell count per vessel was determined. An even cell distribution was applied in the calculation, as previous studies have confirmed that cells distribute uniformly throughout the fixed bed [3].

OPTIMIZATION OF THE MEDIA RECIRCULATION VOLUME TO SUPPORT CELL GROWTH PRE-TRANSFECTION

During the 4 m^2 iCELLis® Nano runs, prior to cell transfection, approximately 8 l of DMEM supplemented with 5% FBS was recirculated throughout the vessel during a 3-day growth period. The glucose levels (measured offline by Vi-Cell) remained >2 g/l throughout the duration of cell growth. With the aim of reducing media use and costs, the recirculation volume was reduced to 4 L for the 3-day growth period prior to transfection. With the reduced volume of media, glucose levels remained >1 g/l and lactate levels remained below 1.5 g/l, comparable to levels measured with an 8 l media recirculation volume (Figure 2). Importantly, there was no measured adverse effect on the rate of cell growth with reduced media volumes, cell counts of 4×10^9 to 8×10^9 were consistently achieved.

TRANSFECTION OF HEK293 CELLS IN THE ICELLIS® NANO

HEK293 cells were transfected using polyethyleneimine (PEI-HCL Max 40,000MW, Poly Sciences Inc.), and a 1:1:1 ratio of the three plasmids (inverted terminal repeat [ITR] vector, AAV rep/cap, and Ad helper plasmid); a ratio of 3:1 PEI:DNA was used. A total of 2.4 mgs of each pDNA was added to 333 ml of serum-free DMEM, an additional 21.6 mls of PEI (1 mg/ml) combined with 333 mls of serum-free DMEM was also added. The PEI plasmid complex was

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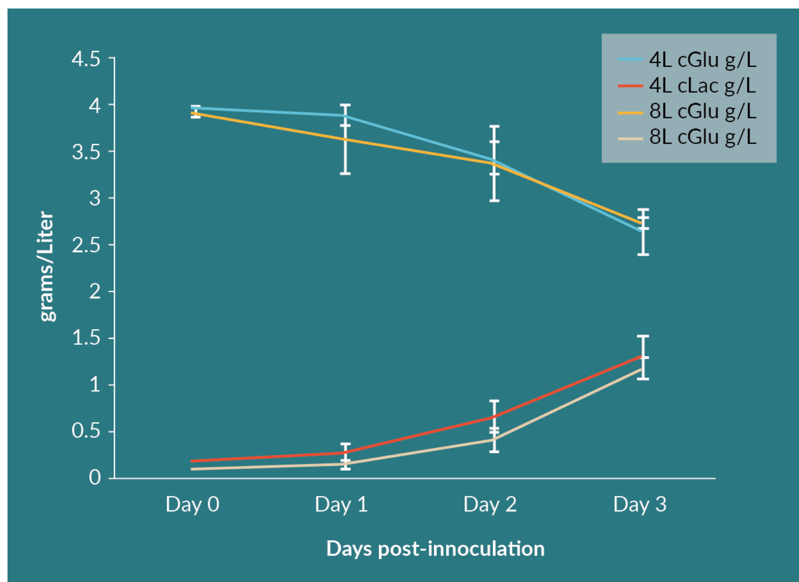
FIGURE 2

Comparison of glucose and lactate levels in the iCELLis® Nano over a 3-day cell growth period using either 4L or 8L media recirculation volumes.



incubated at room temperature for 15 minutes before addition to the bioreactor. Prior to adding the transfection complex to the cells, media circulation was halted and the serum containing media was drained from the iCELLis® Nano vessel. Additionally, during transfection of the cells, the pH control was paused to prevent CO_2 addition to the iCELLis® Nano vessel during the transfection period. An additional 133 mls of fresh serum-free DMEM was added to flush out any residual complex from the sample vessel and lines leading to the iCELLis® Nano vessel. Recirculation with 6 l serum-free DMEM was reinitiated 2 h post transfection, and media samples were taken daily to assess media nutrients and metabolites, culture pH and DO levels. The transfected cells were harvested 96 h post-transfection, both the vessel media and recirculation bulk volume, along with the cell lysate and vessel rinses were pooled prior to clarification and further downstream processing.

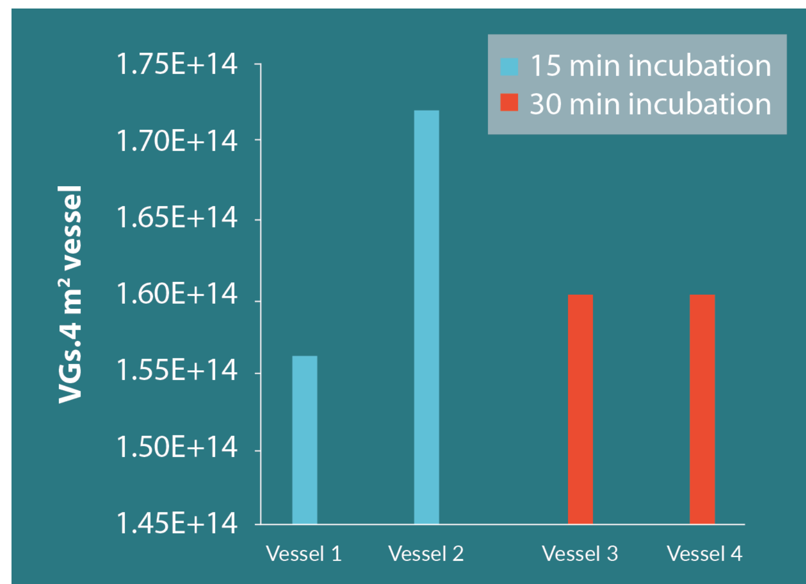
OPTIMIZATION OF PEI: DNA COMPLEX FORMATION

The effect of incubation time on PEI: DNA complex formation and vector yield was evaluated in the 4 m^2 iCELLis® Nano vessels. After addition of the PEI to the DNA mixture, the complex was incubated for 15 min or 30 min prior to adding to the vessel (two vessels per condition). There was an average yield of 1.6 × 10^{14} VGs/Vessel pre-purification for both complex formation times, suggesting that 15 min was enough time to allow for complex formation prior to addition to the cells (Figure 3).

ESTABLISHING OPTIMAL CONTACT TIME OF THE

FIGURE 3

AAV production (VGs / iCELLis® Nano vessel) following a PEI:DNA complex formation time of 15 min or 30 min.



PEI:DNA COMPLEX TO THE CELL SURFACE DURING TRANSFECTION

The optimal time for PEI:DNA complex contact time with the cell surface was first determined in six-well dishes, before further evaluation in the iCELLis Nano. Six-well dishes were seeded with HEK293 cells and transfected with the 3:1 PEI:DNA complex, the complex was allowed to incubate with the cells for 1, 2, 3 or 4 h, before replacing the complex with fresh serum-free media. Transfected cells were harvested 72 h post transfection and vector yields were determined by qPCR. A 5-fold increase in vector yield was measured with the 2 h complex incubation time, compared to incubating the cells for an hour. No significant increase in vector yield was measured when the complex incubation time was extended to 3 or 4 h. The 4 m^2 iCELLis® Nano vessels have a 'high compaction' bed for increased cell densities so it is reasonable to assume that the cells may require longer exposure time for optimal transfection. Cells in the iCELLis® Nano bioreactor were exposed to the PEI:DNA complex for either a 2 or a 3-h exposure time to assess the effects on AAV vector yield. Following exposure of the cells to the PEI:DNA complex, recirculation of fresh media was initiated. A titer analysis of harvest samples was performed to determine vector yields for both conditions; it was determined that 2- and 3-h incubation times resulted in 1.5×10^{14} total VGs/vessel, suggesting that the 2-h exposure time of cells to the PEI:DNA complexes was sufficient to optimally transfect cells in the iCELLis® Nano vessel.

OPTIMIZING MEDIA RECIRCULATION VOLUME TO SUPPORT CELL CULTURE POST-TRANSFECTION

For our initial runs, media recirculation was initiated post transfection with 8 l of serum-free DMEM. With the goal of minimizing downstream processing volumes, reduced recirculation volumes were evaluated. There was a direct correlation in vector yield with media volume, and it was determined that 6 l of media was the lowest working volume that could be used without affecting vector yield. In cases where recirculation volume was reduced to 5 l, vector yields were reduced by as much as three-fold, compared to vector yields achieved using 6-8 l of recirculation media (Figure 4).

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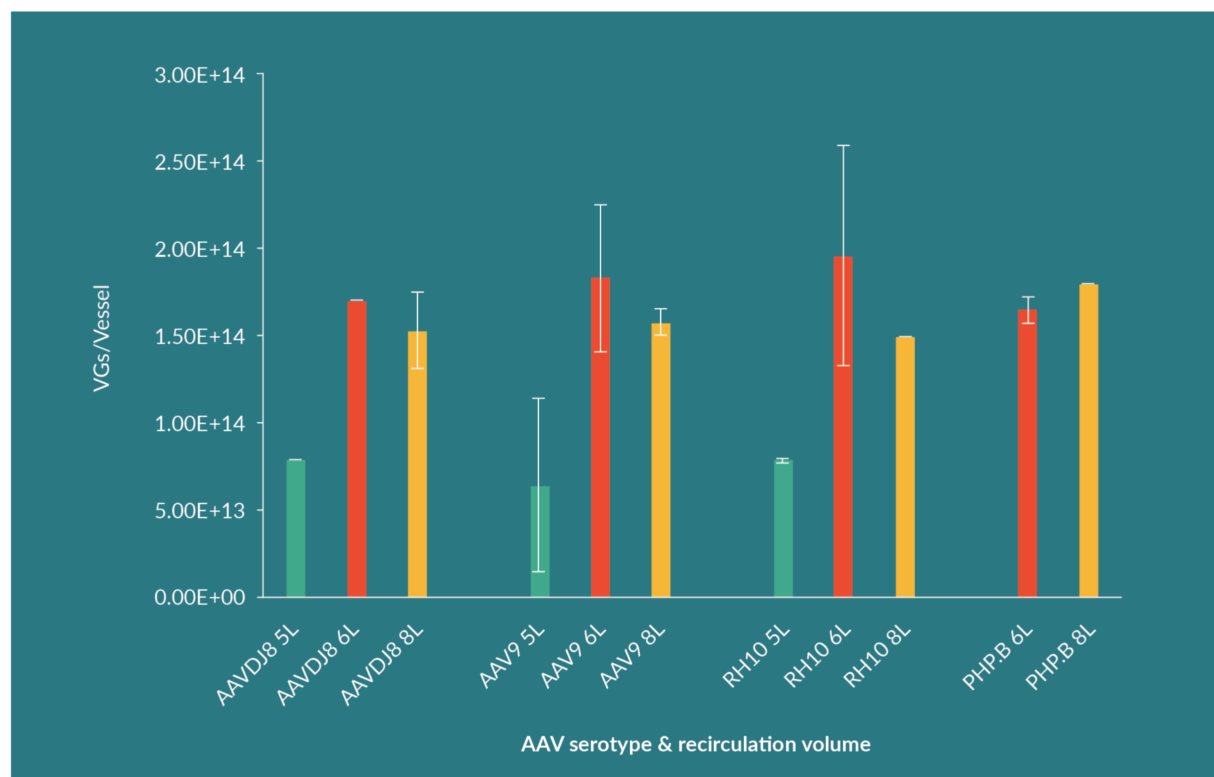
FIGURE 4

The effect of media recirculation volume on vector yield.



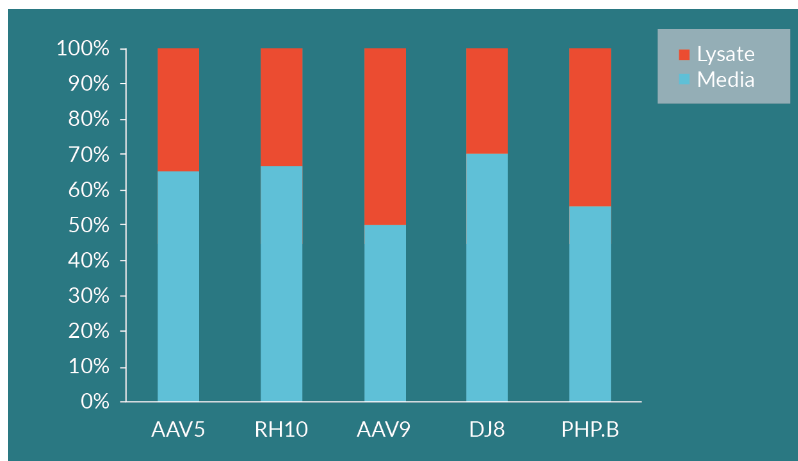
Vector yield (VGs/vessel) for different vectors including AAV9, AAVrh10 and AAV PHP.B is represented as a function of recirculating volume.

CHARACTERIZING AAV VECTOR RELEASE FROM CELLS POST-TRANSFECTION

AAV serotype plays a critical role in how vector fractionates between the intracellular fraction and media during AAV vector production. Vandenberghe *et al.*, showed that in the context of either serum containing or serum free AAV production, serotypes including AAV1, AAV8 and AAV9 can be harvested from the medium of production cultures [4]. In our studies, and in agreement with Vandenberghe *et al.*, approximately half of the AAV9 serotype vector is collected in the harvested media, while the remainder is retained intracellularly. In contrast, for AAVrh10, close to 70% of the vector is released into the media with the remaining 30% retained intracellularly (Figure 5). Determining where a given serotype fractionates during production is critical for designing strategies to harvest vector. Figure 5 shows how various AAV serotypes fractionate between the media and cells, following production in the iCELLis® Nano. For all serotypes evaluated, 50% or greater of the AAV vector fractionated to the media, suggesting that with use of the iCELLis® Nano system, under conditions described here, harvesting both the cellular lysate and media

FIGURE 5

AAV vector distribution following production in the iCELLis® Nano.



The proportion of AAV vector retained intracellularly (red) or released into the media (blue), is represented as a percentage of the total amount of AAV vector produced. n ≥ 2 for each serotype.

will be necessary to maximize vector yields.

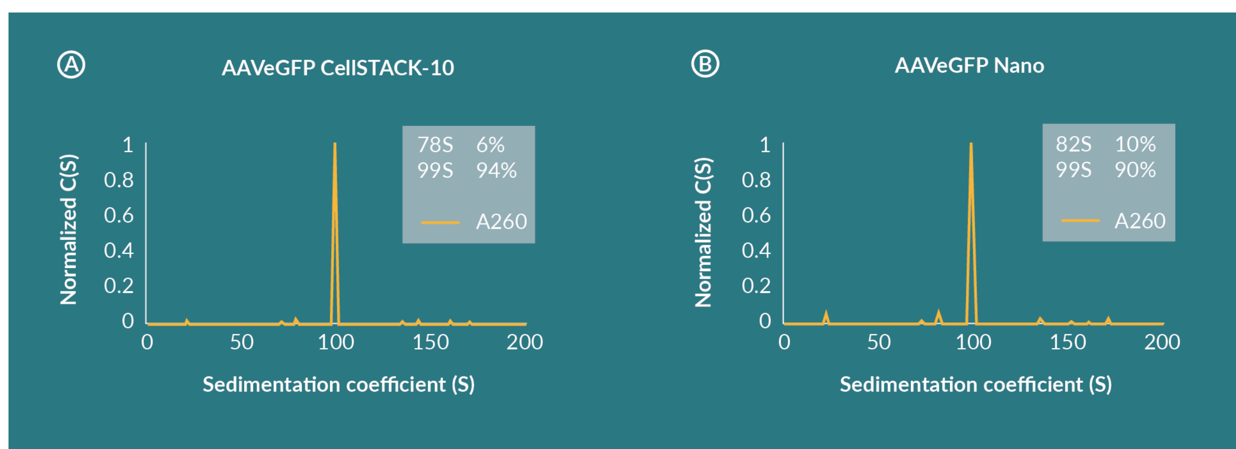
EVALUATION OF AAV VECTOR PRODUCTION FROM THE CORNING

CELLSTACK-10 & THE ICELLIS® NANO BIOREACTOR

The production of AAVeGFP in the iCELLis® Nano 4 m² vessel was evaluated against our standard Corning CellSTACK®-10 AAV production

FIGURE 6

AUC sedimentation distribution plots for an AAV vector produced in either the 10 stack production vessels (A) or the Nano iCELLis bioreactor (B).



The 99S species represents AAV capsids harboring the full vector genome of ~4,000 nucleotides, and the fractional content of this capsid species is similar in vector preparations generated from both production systems; 94% for vector generated in the CellSTACK-10 production vessel **(A)** and 90% for vector generated in the Nano iCELLis bioreactor **(B)**. The 78S and 82S capsid species represent capsids harboring fragmented vector genomes [2].

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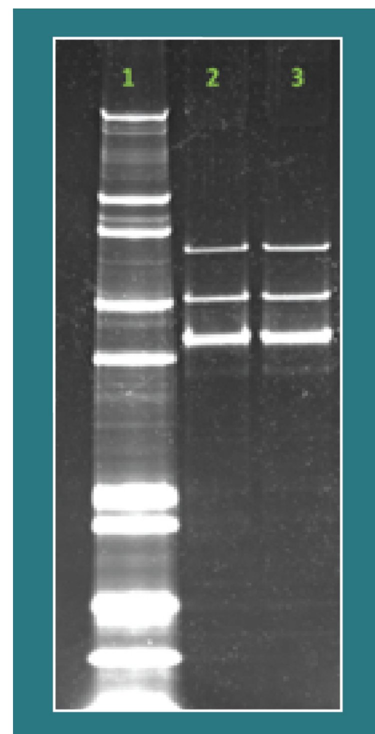
method. For this comparison, 15 CellSTACK®-10 vessels were setup for a total growth surface of 28.5 m^2 and four iCELLis® Nano vessels were used for a total surface area of 16 m^2. Both vessels were inoculated with 10,000 cells/cm^2 of the same stock of HEK293 cells and cell growth proceeded for three days. PEI: DNA complexes were used for transfection as described above. At the time of transfection, the media in the iCELLis® Nano vessel was replaced with serum-free DMEM, in contrast the cell stack remained in DMEM supplemented with 5% FBS. Additionally, at harvest the media and lysed cells were processed from the iCELLis® Nano vessel at 96 h post transfection, while only cell pellets were processed from the planar vessel at 72 h post transfection. The AAVeGFP vector yields at harvest were compared; the cell stack yielded 1.88×10^{13} VGs/m^2 compared to 4.25×10^{13} VGs/m^2 from the iCELLis® Nano vessel, with the caveat that only the intracellular fraction was harvested from the planar vessel. The harvested material from the iCELLis® Nano was clarified and further processed using TFF; AAVeGFP vector from both production platforms was purified using affinity chromatography followed by CsCl density gradient purification [1]. The purified AAVeGFP vector preparations were then compared by analytical ultracentrifugation (AUC), SDS-PAGE for capsid protein ratio, and potency using an in vitro infectivity assay. Figure 6 shows the AUC profiles for both AAVeGFP vector preparations, post CsCl purification, revealing production of similar capsid species from both production systems. The predominant capsid species, in both AAVeGFP preparations sedimented at 99S representing capsids harboring a full vector genome, moreover, there was no evidence of empty particles or capsids harboring fragmented genomes, in either of the AAVeGFP vector preparations [2]. Additionally, SDS-PAGE analysis revealed similar AAV capsid protein ratios for both AAVeGFP vector preparations Figure 7. The infectivity of the AAVeGFP vector preparations was compared by infecting HEK293 cells (1×10^6 VGs per cell) and measuring eGFP expression and vector genome copy number, 72 h post infection. Figure 8 reveals that both AAVeGFP vector preparations yielded similar VGs/cell and levels of eGFP protein, following infection in HEK293 cells, suggesting that both the CellSTACK-10 and iCELLis® Nano bioreactor yielded AAVeGFP vector preparations with comparable potency.

FIGURE 7

SDS-PAGE analysis of AAVeGFP vectors followed by SYPRO RUBY staining.



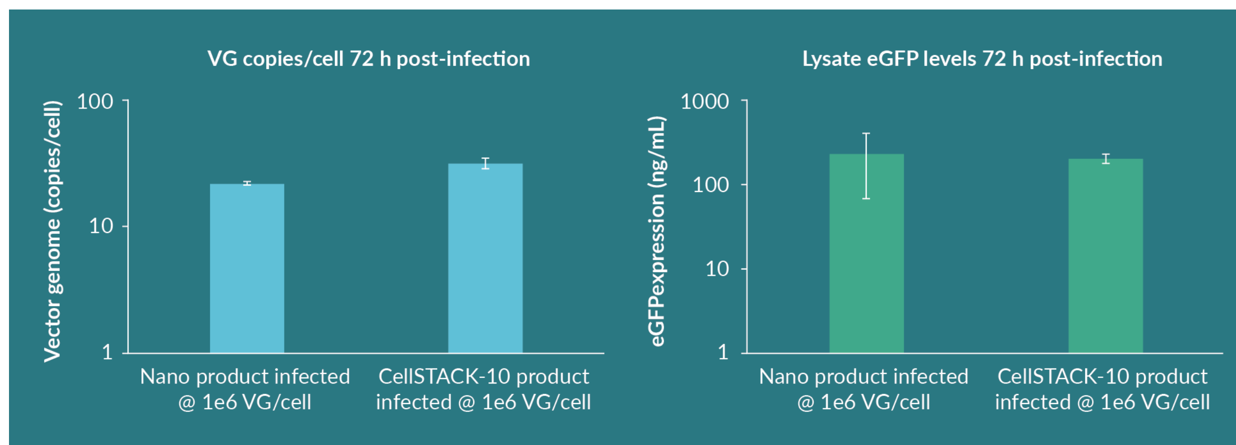
Lane 1: Mark12 Marker (Invitrogen), Lane 2 AAVeGFP vector produced in the CellSTACK-10: 5×10^{10} VGs and Lane 3 AAVeGFP vector produced in the iCELLis® Nano: 5×10^{10} VGs AAV capsid proteins VP1, VP2, and VP3 are present in the correct 1:1:10 ratio.

CONCLUSIONS

We have demonstrated that the iCELLis® Nano bioreactor is an ideal option for AAV vector production at research scale with the potential for scale up to support commercial demand. We show that the AAV production in the iCELLis® Nano generates vector at high yield and comparable potency to vector generated using a more traditional planar vessel. The key advantages of the iCELLis® Nano bioreactor, over CellStack®-10 production, includes constant pH, DO, and temperature control, and improved gas handling and monitoring, ensuring optimal cell viability during the AAV production process. Notably, others have shown, in the context of retroviral vector production, a direct correlation between the oxygen level, the cell growth rate, and

FIGURE 8

Assessment of vector potency.



HEK293 cells were infected with $1x10^6$ VGs/cell of AAVeGFP vector generated from either the iCELLis® Nano or Corning CellStack-10 vessels. Approximately 72 hours post infection, cell lysates were assayed for vector genome copy number (VGs) by qPCR (blue) and for eGFP protein levels by ELISA (green).

the vector titers in the iCELLis® fixed bed [3]. Additionally, we have shown that the iCELLis® production system is compatible with a range of AAV serotypes, including AAV5 and DJ8; all serotypes have consistently produced vector yields in the range of 1-2 × 10^{14} VGs in the iCELLis® Nano 4 m^2, with the potential of producing 2.5 × 10^{16} VGs at the 500 m^2 scale. In the context of clinical dosing this vector yield would support dosing approximately 100,000 patients for an ocular indication, assuming a dose of 1.5 × 10^{11} VGs/eye, the recommended dose for treating LCA2 patients [5]. Alternatively, for a systemic liver directed gene therapy, such as hemophilia Factor IX, dosing of 1,000 patients would be supported, assuming a dose of 5 × 10^{11} VGs/kg [6]. A caveat to these calculations is that losses during purification of vector from the iCELLis® 500+ are not considered, which will vary depending on the process used. Importantly, the optimization experiments described here and by others [7], with the iCELLis® Nano, provides a basis for further development of this system to the larger iCELLis® 500+, a scale that is more compatible with the demands of commercial AAV production.

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: ADHERENT CULTURE METHODS

CHANNEL CONTENT

INTERVIEW

Assessing the future prospects of upstream bioprocessing systems for commercial AAV production

SCOTT A JEFFERS is a Director of Process Development at uniQure LLC. He has been in and out of gene therapy since 1997 when as a graduate student at Purdue University in Dr David A Sanders' lab where he worked on pseudotyping lentiviral and retroviral gene therapy vectors with Ebola virus glycoproteins. He moved out of gene therapy and became a virologist studying SARS virus with Dr Kathryn V Holmes and then made the jump to France were he worked at the Institute Pasteur with Dr Felix Ray elucidating the x-ray crystal structures of the glycoproteins of Rift Valley fever virus and other deadly viruses. He finally broke into industry and back into gene therapy when he worked at Brammer Bio in Florida.

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Q Tell us what you are working on right now.

SAJ: uniQure is a gene therapy company looking for functional cures for liver and CNS diseases. We are currently working on a late-phase hemophilia B project and we also have projects in earlier stages for hemophilia A, Fabry disease, Huntington's disease and spinocerebellar ataxia type 3 (SCA-3). We're a leader in late-phase process development.

In my role, I'm personally responsible for technology transfer and process scaling-up, determining process robustness, and validating the process for commercial manufacturing of our hemophilia B product.

Q Can you outline any particular challenges you encounter in upstream bioprocess development for uniQure's gene therapies?

SAJ: I started working in gene therapy in 1997 as a graduate student at Purdue, and uniQure has been around for 20 years, but gene therapy is still in its very early days. There are still only four approved gene therapy products out there on the market – two *ex vivo* therapies in Kymriah and Yescarta, and two *in vivo* in Luxturna and Zolgensma.

All of those products required developments to be made in recombinant virus production, but the issue is that since the early days of gene therapy, there's been no one expression system to use. I like to compare it to the VHS versus Betamax scenario in the early days of home video recorders when I was a kid: one of these systems is going to be the one that wins out over the other.

The difficulty is that it is still early days and that scenario has yet to play out. Today, there's the adherent HEK system, and there's the suspension HEK system. They're both useful for testing many products at small scale and that testing can be done very quickly. The adherent HEK-293 system, for example, can be used to produce virus very quickly; you can get it into animals very quickly, and you can do rapid, prototype proof of concept experiments.

But the problem is that HEK systems are not going to scale. Scaling is difficult because, with the adherent system, for example, it's a scale-out instead of a scale-up. And that's where I think the biggest difficulty of all has been. For example, in my previous work, I've used adherent HEK-293 in 10 Layer Cell Factories from Corning, and it would take many hundreds – up to a thousand – of these cell factories to be able to dose a single patient in a systemic application.

> "There are still only four approved gene therapy products out there on the market – two *ex vivo* ... and two *in vivo*..."

uniQure has pioneered the use of baculovirus-induced insect cell expression system for the production of our AAV vectors. We think that it is robust and scalable, and we can perform commercial scale manufacturing in our state-of-the-art facility in Lexington, Massachusetts. We currently have a 500-liter system and we're expanding to 2000 liters in the near future.

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We are opening up bigger process development and assay development labs and a pilot plant right now. We use the baculovirus system because it works for our products.

Q Can you dive a bit deeper into the technology and process tools you use – have there been any particular innovations that have stood out for you over recent times, and what future improvements would you like to see?

SAJ: In terms of adherent systems, the iCELLis from Pall is something that's going to boost production and yield considerably. It's a scale-up process of a scale-out process, in essence, which means I can produce more virus in a much smaller space: one iCELLis bioreactor is equal to about 786 CF10 flasks, each of which is a cube of approximately 33 centimeters on a side. If I have to have 786 of those flasks, plus room for them to be manipulated and moved around, that requires a lot of space. The iCELLis, on the other hand, has a footprint of around 1.5 m x 1.5m x 2.5m. So it's a great system, but it is still going to be a scale-out kind of system. Moving forward, I would love to see some of the other fixed bed bioreactors being built out and scaled up to increase our scale of production.

One place where we're going to need to see improvement moving forward is in transfection efficiency. Transfecting all these cells is incredibly expensive at the moment, just in DNA costs alone. If I just had a requirement of one milligram of DNA that I was going to use for each CF10, and I had triple transfection (so I had three plasmids in order to do that), I would have to have more than 2100 milligrams of DNA to transfect the iCellis 500 system. And DNA is really expensive, especially at the GMP level. That is certainly one of the major expenses with mammalian adherent systems, but I would have the same issue in a suspension system. For example, if I need to use 1 ug of DNA per mL of suspension culture and I have three plasmids, I may need between 1.5 to 3 ug of plasmid per mL of suspension culture. This means I may have to use up to *6000* milligrams of DNA with HEK-293 in a 2000-litre reaction. I don't think anyone is planning on doing this large of a scale, and this seems to be unobtainable based on current cost of goods. To me, this is a major advantage of the baculovirus system: I don't have to rely on DNA, and therefore I don't need to rely on outsourced manufacture of a very expensive, critical raw material in order to produce a large-scale batch of vector. Instead, I can bring that in-house, and I can control that critical raw material.

Transfection efficiency is a key area for future improvement. Lipofection is very expensive and the relatively cheaper options, such as Polyethylenimine (PEI), are not as efficient.

If we could get to using microcarriers, like they do with CHO cells, that would be a super advance in the field of adherent mammalian expression for gene therapy.

Q Can you paint us a picture of what you expect the future of commercial AAV-driven gene therapy manufacture will look like in upstream bioprocessing terms? And how will the balance between adherent and suspensions systems develop moving forward?

SAJ: There are multiple systems, of course: for suspension, you've got baculovirus, herpes, transfection of HEK-293 cells, you've got producer cell lines, etc. I'm biased, but I do really think that the baculovirus expression system is going to be the one that goes the furthest. In my honest opinion, suspension will win out over adherent for anything that is systemic, but again, it will require more technology and more drive towards that eventuality.

For smaller-scale production – for delivery to the eye, for instance – I can imagine that you could still use adherent cell culture for the very long term. But I do think the real future is in suspension systems.

Q Finally, what do you and uniQure have coming up through the remainder of 2019 and through 2020? What will be your key goals and milestones over this period?

SAJ: We're continuing our Phase 3 trial for hemophilia B (The HOPE-B trial) – in fact, we just reached our target enrolment, which is great.

We'll dose our first patient in our Huntington's disease program this year. And we'll continue to work on our pipeline and growing our knowledge on how best to produce these gene therapy vectors. That means that, though I now prefer baculovirus, going forward we are

> "...I do really think that the baculovirus expression system is going to be the one that goes the furthest."

 DOI: 10.18609/cgti.2019.134

going to use the best system that's available – the one that's going to get us to our targets.

I'm excited to work with uniQure and to see the growth in the gene therapy industry in general. I think we are at a time where in medicine where we're not just going to be just treating patients; we're going to be providing functional cures for them for some pretty horrible diseases.

Luckily, I don't have a child with a rare disease, but I do think often about the children that have rare diseases and how we're changing their lives. That truly is something that brings me into work every day – having the ability and the opportunity to make a difference.

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INTERVIEW with Kasey Kime, Senior Manager of Regulatory Affairs Clinical and Compliance, and **Michael Brewer**, Director and Global Principal Consultant, Regulatory at Thermo Fisher.

"The quality of raw materials needs to be considered according to the stage of development of the cell or gene therapy..."

Enabling cell & gene therapy raw materials standardisation and regulatory compliance

Kasey Kime, has 15 years of global quality and regulatory affairs experience in Life Sciences. She is part of Thermo Fisher Scientific's regulatory affairs division and is overseeing regulatory compliance of technologies developed for cell and gene therapy applications. Her areas of expertise include raw material risk assessment for biopharmaceutical development and regulatory compliance of instruments and consumables developed for automating cell and gene therapy manufacturing. Kasey holds a Bachelor's degree in Medical Laboratory Science and postgraduate degrees in both Microbiology and Quality Systems Management.

Michael Brewer is the Director, Global Principal Consultant, Regulatory for the BioProduction Division (BPD) at Thermo Fisher Scientific. In this role, Michael is responsible for providing global support to BioProduction customers and serving as the regulatory thought leader and expert across all technology areas within BPD. Prior to moving to this role, he led the team responsible for product applications including Microbiology, Analytical Sciences and Quality control. The products are fully integrated, solutions for

Glycan profiling, Bacterial and Fungal identification, Mycoplasma and Viral detection and host cell DNA and protein quantitation. Michael has over 30 years experience in the Biopharma industry, including, Scios, Synergen and Amgen in a variety of roles including Discovery Research, Analytical Sciences and Quality Control. Prior to joining Thermo Fisher Scientific, he led a group at Amgen that developed qualified, validated and implemented molecular methods for host cell DNA quantitation, contaminant (Mycoplasma, Virus and Bacteria) detection, contaminant identification, strain typing and genotypic verification of production cell lines.

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Q What are the key elements to consider when selecting reagents and media at an early stage of R&D?

KK: Performance is always going to be very important in early R&D although quality and safety should also be considered. The quality of raw materials needs to be considered according to the stage of development of the cell or gene therapy, acknowledging the quality profile does evolve during clinical development. However, it is still important to assure patient safety even in early clinical development.

MB: In addition, it's important to choose reagents, components of your process, media, etc. early on that will meet the most rigorous regulatory expectations that will come later in development. This avoids the need to make changes, justify those changes and go through the change control process as you get closer to the clinical and commercial stages.

Q Delving deeper on the topic of supporting documentation and certification, can you explain the specific utility and benefits of the various options in this regard? (RSFs, DMFs, COAs, COOs)

KK: It is important for cell and gene therapy developers to be aware of any safety risks within media or reagent products especially, if they contain biological-derived components within their formulation and/or manufacturing process. CGT developers should begin assessing raw materials for suitability in manufacturing by reviewing the supplier's COA and COO. Both of these documents will provide the end user with the data to begin further risk assessments.

Master Files can be useful in regions that support master file processes for raw materials such as USA, Canada and Japan. Master Files are popular for suppliers as they limit the amount of confidential information disclosed to the end users. However, many regions do not support master file processes for raw materials and often the information within the master file is also desired to be disclosed to the end user. In these situations, suppliers may provide Regulatory Support Files (RSFs) under CDA to clinical customers. The RSF is likely to contain a CMC-style summary of the same data that is within the Master File. Often it will provide qualitative levels of components rather than quantitative levels to protect confidentiality concerns on media/reagent formulations.

Japan has a very unique raw material certification process that enables media and reagent suppliers to submit evidence to the PMDA that their raw materials comply with Japanese Standard for Biological Ingredients (SBI). If the PMDA approves the raw materials meet the requirements as per the SBI, a certificate is issued to the supplier. The supplier can share the certificate of SBI compliance with developers in preclinical phases so they can make informed raw material choices thereby helping to assure correct raw material choices early in the process. This process reduces the burden for raw material risk assessment on the developer, the supplier and the regulatory agency.

Q What degree of importance do you place on AOF certification and why?

KK: Animal origin free is definitely the goal. It is desirable because it helps to reduce adventitious agent risk concerns which are still one of the main regulatory filing deficiencies for CGT customers using biological-derived reagents. By having a certified animal origin free product you remove the need to prove viral safety of biologically-derived components. However, we still need regulatory agencies to agree on the definition of AOF and the levels of AOF such as primary level or secondary level or beyond. Suppliers and CGT manufacturers

> "By having a certified animal origin free product you remove the need to prove viral safety of biologically-derived components." -KK

need to ensure the supply chain involved in the manufacturing of media and reagents destined for use in CGT, are educated and understand the need for accurate AOF statements for their components.

"...choose solutions that have been successfully validated and implemented into a manufacturing process similar to yours." -MB

Q What differences do you see between the USA and Europe in terms of the degree of importance developers and manufacturers place on key certifications and compliance with various standards/guidelines?

KK: I notice that both the USA and EU expect raw and ancillary materials to comply with the associated pharmacopeia chapters as applicable. In the USA this is general chapter USP <1043> while in the EU this is Ph Eur 5.2.12. Both regions want well characterized, high quality/GMP products intended to be used as raw/ancillary materials in CGT manufacturing processes. Supplier relationships are very important. Developers want to ensure a good relationship with their suppliers of critical raw materials to enable timely answers to questions posed to them by regulatory agencies. They want to ensure their supplier will work with them to obtain answers to unusual requests or modify products/testing as and when required.

Q Why do these variations exist, and how do you go about identifying an optimal approach in each region and overall?

KK: At Thermo Fisher we have a global regulatory affairs team that continuously monitor the regulatory landscape for new and emerging regulations and assess the impact of these on our products and services. Our general approach is to incorporate both customer and global regulatory agency expectations into our product requirements. We are frequently audited by customers and have direct dealings with agencies on raw/ancillary material CMC matters so we do get a lot of useful feedback to ensure we have global acceptance of our products. This global approach is important for developers using Thermo Fisher products in clinical trials in multiple countries.

For some regions, such as Japan, our products are already designed to meet the SBI requirements so in this case we apply for the SBI certificate because this is a regional expectation but it is also of global value to customers looking to perform clinical trials in Japan.

"One of the things we do at Thermo Fisher is to ensure we factor in global regulatory requirements for raw and ancillary materials into our product design process." -KK

Q Standardization of raw material quality testing is a major priority for the sector – how is Thermo Fisher Scientific helping to drive this?

KK: Our global Regulatory Affairs team help support customer and regulatory inquires on Thermo Fisher reagents and media in CGT manufacturing and actively contribute to standards development and regulatory initiatives for raw/ancillary materials. Our R&D and Product Management teams also actively contribute to industry working groups addressing topics such as the importance of standardization of raw materials.

One of the things we do at Thermo Fisher is to ensure we factor in global regulatory requirements for raw and ancillary materials into our product design process. New and emerging requirements are also considered in our product design because we know the most suitable raw and ancillary materials are those that are designed for this purpose.

Publication of the new ISO working draft for Ancillary Materials present during the production of cells and cellular therapeutic products is also highly anticipated. Once finalized, this will represent globalized guidance to suppliers and developers on best practices to ensure consistent, high quality and safe raw/ancillary materials.

Q Addressing the issue of regional differences between regulatory requirements relating to changing raw materials, what for you is the best strategic approach to this challenge?

KK: One of the questions developers need to ask themselves is whether they need to consider a global raw material approach early on. It is true there are regional differences and some regions may have more detailed requirements

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> "Partnering with a vendor that has experience in guiding qualification, validation and regulatory filings that have been accepted can streamline the implementation process and reduce the risk of extended regulatory review." -MB

on particular characterization or viral safety expectations than others. In some regions, detailed raw material requirements may not yet be published or there may be multiple interpretations leading to confusion around the regulatory requirements. For these reasons developers should aim to choose well characterized, high quality raw materials intended for use in cell and gene therapy manufacturing processes that meet the current regulatory guidance's in the major markets (such as USA, Europe and Japan). The use of such materials should ensure regulatory acceptance and avoid the need to make changes to materials due quality or regulatory deficiencies.

Q What is your advice to cell therapy developers in terms of selecting and optimizing their contaminant and impurity testing regimes with current regulatory requirements in mind?

MB: Although there are many potential options available, my advice is to choose solutions that that have been successfully validated and implemented into a manufacturing process similar to yours. Partnering with a vendor that has experience in guiding qualification, validation and regulatory filings that have been accepted can streamline the implementation process and reduce the risk of extended regulatory review.

Q Can you go deeper on the benefits rapid mycoplasma testing in particular can bring to cell therapy manufacture?

MB: As many cell-based therapies have limited shelf life, a rapid result from the required Mycoplasma test is needed to ensure the product is free of Mycoplasma prior to patient treatment. In particular, some qPCR-based Mycoplasma tests have been shown in validation to be sensitive, specific and reliable enough to meet regulatory expectations for Mycoplasma testing. Ask for examples of successful validations and regulatory acceptances as part of your due diligence in selecting a solution for your testing needs. I recommend selecting a vendor partner that has the support team and experience in place to support your implementation process.

Q Can you pick out some key considerations and specific issues when designing environmental testing programs across different regulatory jurisdictions?

MB: My advice would be to start by reviewing USP <1116> Microbiological Control and Monitoring of Aseptic Processing Environments. This chapter is quite comprehensive and provides a great foundation for sponsors to understand the process and how it could be applied in their manufacturing environment. Engaging an experienced consultant can also be an advantage when implementation needs to be accelerated.

AUTHORSHIP & CONFLICT OF INTEREST

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COMMERCIAL INSIGHT: OCT 2019

Commercial insight: cell and gene therapy

Providing a critical overview of the sector's commercial developments – M&As, licensing agreements & collaborations, financial results, IPOs and clinical/regulatory updates, with commentary from our Expert Contributors.

CELL THERAPY

Mark Curtis. Financial Portfolio Manager, Emerging Technologies, Lonza AG, Switzerland

It was a busy month for financings with Tmunity, ArsenalBio, Arcellx, and Adicet all completing financings of $75 million or more. Tmunity has been focused on building its team and in-house manufacturing capabilities. In addition to its existing capacity at UPenn it will add a new facility in the coming years. A series B round of $75 million will help the company fund multiple programs that are through IND as they progress towards mid-stage clinical studies. Arcellx will deploy its $85 million A round to develop next-generation T-cell therapies, initially targeting BCMA for multiple myeloma. In other news ElevateBio launched its second company, HighPassBio, to develop a cell therapy from Fred Hutch.

GENE THERAPY

Richard Philipson. Chief Medical Officer, Trizell Ltd, UK

Announcements of positive clinical trial data from Audentes Therapeutics and Rocket Pharmaceuticals provide a boost to research in x-linked myotubular myopathy (XLMTM) and Fanconi anemia (FA) respectively. Both diseases present in early life with life threatening, life-limiting clinical manifestations; XLMTM has no effective treatment and FA can only be treated with bone marrow transplantation from an unaffected donor, with its attendant risks. Rocket's FA treatment is particularly noteworthy as although it requires the use of transplanted autologous, gene corrected hematopoietic stem cells, it does

not require any conditioning regimen. It's been a good month for Rocket, as it has also received clearance of its IND application by FDA for its lentiviral vector-based gene therapy for the treatment of Pyruvate Kinase Deficiency, with the first clinical trial due to start enrolment of 6 patients, with completion scheduled for March 2023.

CLINICAL/REGULATORY

CABALETTA BIO'S CAART THERAPY RECEIVES IND CLEARANCE FROM THE FDA

Cabaletta Bio, a clinical-stage biotechnology company developing engineered T cell therapies to treat B cell-mediated autoimmune diseases, has received Investigational New Drug (IND) clearance from the FDA to initiate a first-in-human clinical trial of desmoglein 3 chimeric autoantibody receptor T cells (DSG3-CAART) in patients with mucosal pemphigus vulgaris (mPV).

mPV is a rare, B cell-mediated chronic, autoimmune disease that causes painful blisters and sores on mucous membranes of affected patients, leading to severe and sometimes debilitating and life-altering effects. It is caused autoantibodies that target desmoglein 3 (DSG3) in the mucosal membranes and these autoantibodies disrupt structural proteins within the mucosa that connect with other proteins to enable mucosal cells to connect with each other.

Chimeric AutoAntibody Receptor (CAAR) T cells are designed to selectively bind and eliminate only disease-causing B cells, while sparing the normal B cells that are essential for human health. CAAR T cells are based on the chimeric antigen receptor (CAR) T cell technology developed at the University of Pennsylvania (UPenn). While CAR T cells typically contain a CD19-targeting molecule, CAAR T cells express an autoantibody-targeted antigen on their surface. The co-stimulatory domain and the signaling domain of both a CAR T cell and a CAAR T cell carry out the same activation and cytotoxic functions. Thus, Cabaletta's CAARs are designed to direct the patient's T cells to kill only the pathogenic cells that express disease-causing autoantibodies on their surface, leading to complete and durable remission of disease while sparing all other B-cell populations that provide beneficial immunity from infection.

DSG3-CAART is designed to selectively target and eliminate B cells expressing autoantibodies specific for DSG3, while preserving healthy B-cell immune function. DSG3-CAART has the potential to generate persistent complete remission off therapy while avoiding

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the adverse effects of chronic and generalized immunosuppression. The trial which is expected to enroll the first patient in 2020, will assess the safety and tolerability of DSG3-CAART in mPV patients.

Cabaletta was founded by UPenn scientists, Dr Michael Milone, Dr Aimee Payne and Dr Steven Nichtberger. Cabaletta has an exclusive global licensing agreement and multiple research agreements with the UPenn to develop the CAAR T technology to treat B cell-mediated autoimmune diseases.

Dr Nichtberger, CEO of Cabaletta commented:

"The FDA's clearance of our IND for DSG3-CAART is an important milestone for patients with mPV and the first IND clearance for a product candidate from our Cabaletta Approach to selective B cell Ablation (CABA™) platform. DSG3-CAART is the first of several CAAR T cell product candidates in our announced pipeline, which includes product candidates targeting patients with MuSK myasthenia gravis, the mucocutaneous form of pemphigus vulgaris (PV), and hemophilia A patients with inhibitors to factor VIII therapy."

In additional news this month, Cabaletta has presented positive data from its preclinical *in vitro* study which tested CAART cells developed against anti-muscle-specific tyrosine kinase (MuSK) antibody-expressing target cells to treat MuSK-associated myasthenia gravis (MG). Data was presented at the American Neurological Association (ANA) 2019 Annual Meeting in October in St. Louis. MuSK-CAART is Cabaletta's second product candidate.

AVROBIO'S GENE THERAPY RECEIVES FDA'S ORPHAN DRUG DESIGNATION

The US Food and Drug Administration (FDA) has granted orphan drug designation to AVROBIO's investigational lentiviral-based gene therapy, AVR-RD-02, for the treatment of Gaucher disease.

Gaucher disease is caused by an inherited deficiency of the enzyme glucocerebrosidase and causes the build-up of the fatty substance glucosylceramide in numerous tissues and organs. AVR-RD-02 targets the faulty gene via a modification of the patient's own hematopoietic stem cells. A one-time treatment, it is delivered via infusion and expected to sustain a long-term supply of the endogenous enzyme. It is hoped that the treatment will be able to replace the current enzyme replacement course of treatment.

The company is now actively recruiting patients for its Phase 1/2 clinical trial of AVR-RD-02 in Canada and the study aims to evaluate the safety and efficacy of the therapy in patients with Type 1 Gaucher disease.

The therapy is the company's second targeting lysosomal storage disorders.

Orphan-drug designation is granted by the FDA to drugs and biologics which are intended for the safe and effective treatment, diagnosis or prevention of rare diseases or conditions that affect fewer than 200,000 people in the USA.

Dr Birgitte Volck, President of Avrobio's R&D commented:

"Under the existing standard of care, patients with Gaucher disease are bound to a lifelong infusion schedule of enzyme replacement therapies, and still experience painful and progressive symptoms such as debilitating musculoskeletal pain and fatigue. Orphan-drug designation recognizes the unmet need of populations with rare diseases like Gaucher where AVROBIO strives to transform lives by addressing the underlying cause of the disease with a single dose of gene therapy."

News that AVROBIO's lentiviral-based gene therapy for Gaucher disease has received Orphan Drug Designation from FDA is not particularly surprising, given the rarity of the condition, which affects 1 in 50,000 to 100,000 people in the general population. Nevertheless, it marks an important milestone for AVR-RD-02, which is currently being evaluated for safety and pharmacodynamic effects in a Phase 1/ 2 clinical trial in approximately 8 to 16 subjects at a single center in Calgary, Canada. AVR-RD-02 is being developed for the type 1 form of Gaucher disease, which typically spares the central nervous system; manifestations of the condition include hepatosplenomegaly, anemia, thrombocytopenia, lung disease and bone abnormalities. The treatment requires stem cell harvest, bone marrow conditioning and transplant of transduced $CD34^+$ cells – a very significant undertaking for patients who already have an alternative approved therapy in VPRIV, indicated for long-term enzyme replacement therapy (ERT) in patients with type 1 Gaucher disease. AVR-RD-02 will therefore likely have to demonstrate convincing long-term 'cure' (freedom for ERT requirement) to be a viable treatment option. – Richard Philipson

FDA GRANTS IND CLEARANCE TO ROCKET'S GENE THERAPY FOR PYRUVATE KINASE DEFICIENCY

Rocket Pharmaceuticals has announced that it has received FDA's IND clearance for RP-L301, the investigational lentiviral vector-based gene therapy for pyruvate kinase deficiency (PKD).

The gene therapy was licensed from the Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) and Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD).

The IND acceptance follows the recent clearance of the Investigational Medicinal Product Dossier (IMPD) for RP-L301 by the Spanish Agency for Medicines and Health Products (AEMPS) in September. The trial will be led by principal investigator Dr Sandeep Soni at the Stanford University School of Medicine.

The global Phase 1 clinical trial will investigate the safety, tolerability and preliminary clinical efficacy of a single-administration of RP-L301 in PKD patients.

PKD is a rare red blood cell disorder and is caused by a mutation in the PKLR gene. PKLR gene codes for the pyruvate kinase enzyme, a

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key component of the red blood cell glycolytic pathway, mutation of which results in increased red cell destruction leading to severe anemia. Currently available treatments include splenectomy and red blood cell transfusions, which are associated with immune defects and chronic iron overload.

Lucile Packard Children's Hospital Stanford will serve as the lead site in the USA for adult and pediatric patients. Hospital Infantil Universitario Niño Jesús will serve as the lead site in Europe for pediatrics and Hospital Universitario Fundación Jiménez Díaz will serve as the lead site in Europe for adult patients.

RP-L301 was shown to reduce anemia in preclinical models, where at least 20–30% of bone marrow progenitor cells were genetically corrected.

Kinnari Patel, COO and Head of Development of Rocket commented:

"In less than 12-months, four Rocket-sponsored INDs received clearance from the FDA. The RP-L301 IND marks an important milestone as it is the first global Phase 1 study for Rocket in the USA and EU. This achievement would not have been possible without the team's dedication and commitment to bringing first and best in class curative gene therapies to patients as quickly as possible".

ELEVATEBIO LAUNCHES HIGHPASSBIO TO ADVANCE TARGETED T-CELL IMMUNOTHERAPIES

Cambridge-based biotechnology company ElevateBio has launched HighPassBio to advance novel targeted T-cell immunotherapies. The company's lead product candidate which is currently being tested in a Phase 1 clinical trial, is an engineered T-cell receptor (TCR) T-cell therapy for HA-1 expressing tumors. It is designed to treat and potentially prevent relapse of leukemia following hematopoietic stem cell transplant (HSCT).

TCR is a protein present on T cells that helps them to recognize leukemia cells. HA-1 is a protein that is present on the surface of some peoples' blood cells, including leukemia. HA-1 T-cell immunotherapy enables genes to be added to the donor cells to make them recognize HA-1 markers on leukemia cells.

The product and the research that led to the immunotherapy development were developed by researchers at Fred Hutchinson Cancer Research Center. HighPassBio's scientific founder is Dr Marie Bleakley, pediatric oncologist and stem cell transplant physician at Fred Hutchinson Cancer Research Center.

The ongoing Phase 1 trial has treated initial patients and is now recruiting adult and pediatric leukemia patients who have undergone blood and marrow transplantation but whose leukemia or related conditions has relapsed.

David Hallal, Chairman and CEO of ElevateBio commented:

"We look forward to leveraging our centralized industry-leading cell and gene therapy process development

and manufacturing capabilities while working closely with Dr Marie Bleakley and her team, to accelerate their impressive work through clinical development with the goal of serving patients who have no other treatment options. Additionally, we will explore this approach as a potential treatment for other diseases that are treated by stem cell transplants."

HIGHPASSBIO LAUNCHES SECOND COMPANY

ElevateBio was founded by a group of industry experts to help incubate companies and develop drug products through a collaborative business model with shared manufacturing resources. Its second company, HighPassBio, will develop a T-cell therapy from Fred Hutch that is designed to target an antigen associated with relapse following bone marrow transplant. With access to development and manufacturing resources at ElevateBio's BaseCamp, HighPass should be able to expedite evaluation of the therapy in human clinical studies.- Mark Curtis

EMA APPROVES REFINED COMMERCIAL MANUFACTURING SPECIFICATIONS FOR BLUEBIRD BIO'S GENE THERAPY

The European Medicines Agency (EMA) has approved the refined commercial drug product manufacturing specifications for bluebird bio's gene therapy Zynteglo, for treating patients with transfusion-dependent beta-thalassemia.

Zynteglo, previously known as lentiglobin, is a cell-based gene therapy where autologous CD34$^+$ cells from patients are transduced ex vivo with a lentiviral vector encoding βA-T87Q-globin gene. Following transplantation of these gene-corrected stem cells into patients, patients are monitored for the production of gene therapy-derived hemoglobin (Hb) which increases Hb levels.

Transfusion-dependent beta-thalassemia (TDT) is an inherited blood disorder caused by a mutation in the beta-globin chain resulting in ineffective red blood cell production. Anemia caused by TDT is corrected by blood transfusions, however, regular blood transfusions leads to iron overload.

The refined commercial drug product specifications support the efficacy and safety profile of Zynteglo and will give patients the best opportunity for clinically meaningful outcomes consistent with the results that were foundational to the conditional marketing authorization in the European Union. Zynteglo provides hope for a category of TDT patients above 12 years, those who do not have a β0/β0 genotype for whom hematopoietic stem cell transplantation is appropriate, but a human leukocyte antigen (HLA)-matched related HSC donor is not available.

The conditional marketing authorization was supported by efficacy, safety and durability data from

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the Phase 1/2 HGB-205 study and the completed Phase 1/2 Northstar (HGB-204) study as well as available data from the ongoing Phase 3 Northstar-2 (HGB-207) and Northstar-3 (HGB-212) studies, and the long-term follow-up study LTF-303.

Zynteglo-related non-serious adverse events reported were hot flush, breathing difficulty, abdominal pain, pain in extremities and non-cardiac chest pain. Thrombocytopenia was one serious adverse event reported which was related to Zynteglo.

ATLAS VENTURE LAUNCHES KORRO BIO TO ADVANCE RNA EDITING TECHNOLOGY

Atlas Venture has launched Korro Bio to lead the rapidly advancing field of RNA editing. The company was co-founded and incubated by Atlas, with additional funding from New Enterprise Associates.

The company is advancing a proprietary platform designed to selectively edit messenger RNA and recode specific codons to effect changes in protein structure and function across multiple tissues.

Gene editing industry pioneers Dr Nessan Bermingham and Dr Andrew Fraley formed Korro Bio in 2018 and the foundational technology behind it originated from the pioneering research in the laboratory of company co-founder Dr Josh Rosenthal from the Marine Biological Laboratory (MBL) in Woods Hole, Mass., an affiliate of the University of Chicago. Dr Rosenthal's discoveries in RNA editing are based on nucleotide deamination, an endogenous process for modifying RNA function that is common to all multicellular organisms.

Korro Bio's proprietary RNA-editing approach leverages endogenous human-expressed RNA-editing enzymes in the family of adenosine deaminase acting on RNA (ADAR).

The ADAR platform at Korro Bio has multiple potential advantages over existing gene editing platforms, including the ability to utilize both endogenous or exogenous effector proteins; the potential for highly efficient and allele-specific RNA editing; the use of multiple delivery technologies; and the potential for titratable, repeat dosing.

The company is prioritizing multiple therapeutic indications where safe and targeted editing of messenger RNA using its ADAR-based platform is poised to provide unique benefits over other modalities in development, including gene therapy and gene editing approaches.

Dr Bermingham commented:

"The field of nucleic acid editing is progressing rapidly, and new discoveries are creating the opportunity to harness endogenous human biology and develop compelling new therapies. Korro Bio was established as the leader to watch in this space, supported by strong science, proprietary intellectual property and a team of accomplished experts to drive progress forward. We are thrilled to partner with NEA, which has been with Atlas at the forefront of evaluating and funding new gene-editing technologies."

AUDENTES' AAV GENE THERAPY PROVIDES HOPE FOR MYOPATHY PATIENTS

San Francisco-based Audentes Therapeutics, a development-stage biotechnology company developing new therapies for patients with rare muscle diseases using AAV gene therapy technology, has presented new positive data from its clinical trial, ASPIRO, which is evaluating AT132 in patients with X-Linked Myotubular Myopathy (XLMTM).

Data presented at the 24th International Annual Congress of the World Muscle Society by Dr James J Dowling showed that the study had yielded encouraging efficacy and safety profile.

XLMTM is a serious, life-threatening, rare neuromuscular disease that is characterized by extreme muscle weakness, respiratory failure, and early death. Mortality rates are estimated to be 50% in the first 18 months of life, and for those patients who survive past infancy, there is an estimated additional 25% mortality by the age of 10. XLMTM is caused by mutations in the MTM1 gene that lead to a lack or dysfunction of myotubularin, a protein that is needed for normal development, maturation, and function of skeletal muscle cells. The disease affects approximately 1 in 40,000 to 50,0000 newborn males.

AT132 is an AAV8 vector containing a functional copy of the MTM1 gene, for the treatment of XLMTM. The preclinical development of AT132 was conducted in collaboration with Genethon. AT132 has been granted Regenerative Medicine and Advanced Therapy (RMAT), Rare Pediatric Disease, Fast Track, and Orphan Drug designations by the US Food and Drug Administration (FDA), and Priority Medicines (PRIME) and Orphan Drug designations by the European Medicines Agency (EMA).

Treated patients across both dose cohorts show significant reductions in ventilator dependence and the progressive attainment of developmental motor milestones, suggesting that AT132 has the potential to deliver transformative benefit to patients and families living with XLMTM.

The newly reported data include safety and efficacy assessments as of the August 7, 2019 data cut-off date for 12 patients enrolled in the ASPIRO dose escalation cohorts. The data includes 48 weeks or more of follow-up for seven patients enrolled in Cohort 1 ($1x10^{14}$ vector genomes per kilogram (vg/kg); six treated and one untreated control) and 24–48 weeks of follow-up for five patients in Cohort 2 ($3x10^{14}$ vg/kg; four treated and one untreated control). Key assessments include neuromuscular function as assessed by the achievement of motor milestones and improvement in CHOP INTEND score, and respiratory function as assessed by reduction in ventilator dependence and improvement in maximal inspiratory pressure (MIP). Today's presentation does not include new muscle biopsy data.

Patients receiving AT132 have achieved significant and durable reductions in ventilator dependence, an endpoint considered to be closely correlated with morbidity and mortality in XLMTM patients. To date,

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the first seven patients treated (all six treated patients in Cohort 1 and the first patient treated in Cohort 2) have achieved ventilator independence. All treated patients continue to show gains in neuromuscular function, with the first seven patients treated achieving the ability to rise to a standing position, or walk.

AT132 has been generally well-tolerated and has shown a manageable safety profile across both dose groups.

The company is now aiming to complete the enrollment and follow-up of patients in the ASPIRO pivotal expansion cohort, designed to confirm the safety and efficacy profile of AT132 at a dose of $3x10^{14}$ vg/kg, and preparations for filing of a Biologics License Application (BLA) for AT132 in the USA planned in mid-2020 and filing of a Marketing Authorization Application (MAA) in Europe planned for the second half of 2020.

Data emerging from Audentes Therapeutics' Phase 1/2 study in x-linked myotubular myopathy (XLMTM) provide very encouraging evidence for the efficacy of the company's AAV8-based therapy. Of 12 patients enrolled and reported in the presentation at the World Muscle Society Annual Congress, 7/10 treated patients have achieved ventilator independence and all treated patients show evidence of improvements in neuromuscular function. Part 1 of the study – the dose escalation phase (N=14) – is now complete and Part 2 – the pivotal expansion cohort (N=8) – is underway, using the higher of the two doses studied in Part 1. The company's plan is to submit applications for approval in both the US and EU in 2020; if these encouraging data are replicated in the expansion cohort, with persistence in benefit, then this could be a transformational treatment for a condition with 50% mortality in the first 18 months of life.- Richard Philipson

LICENSING AGREEMENTS & COLLABORATIONS

BLUEBIRD BIO PARTNERS WITH NOVO NORDISK TO DEVELOP *IN VIVO* GENOME EDITING CANDIDATES

bluebird bio has entered into a research collaboration with Novo Nordisk to develop next-generation *in vivo* genome editing treatments for genetic diseases, including hemophilia. During the three-year partnership, the companies will collaborate to identify a

gene therapy candidate that could treat hemophilia A so that patients could be free of factor replacement therapy.

The research collaboration will use bluebird bio's proprietary mRNA-based megaTAL™ technology that could specifically and efficiently silence, edit or insert genetic components.

MegaTALs are a single-chain fusion enzyme that combines the natural DNA cleaving processes of Homing Endonucleases with the DNA binding region of transcription activator-like (TAL) effectors. TALs are easily engineered proteins that recognize specific DNA sequences. This protein fusion architecture allows the generation of active and specific nucleases that are compatible with all current viral and non-viral cell delivery methods.

Novo Nordisk is specialized in developing therapies for hemophilia and the new research collaboration will initially focus on correcting FVIII-clotting factor deficiency, with the potential to explore additional therapeutic targets.

Philip Gregory, bluebird bio's CSO commented:

"bluebird has made tremendous progress on enabling an **in vivo** *gene editing platform based on our megaTAL technology, including important advances in high-quality mRNA production and purification," he said. "We believe this technology has the potential to create a highly differentiated approach to the treatment of many severe genetic diseases. Moreover, we are thrilled to be able to combine this new platform technology with Novo Nordisk's deep expertise in hemophilia research and therapeutics. We believe this collaboration will move us toward our shared goal of recoding the treatment paradigm and substantially reduce the burden of disease for patients with factor VIII deficiency."*

GSK TO COLLABORATE WITH LYELL IMMUNOPHARMA TO DEVELOP CANCER CELL THERAPIES

GlaxoSmithKline plc has signed a five-year collaboration with Lyell Immunopharma, a San Francisco based biotechnology company, to develop next generation cancer cell therapies. The collaboration will apply Lyell's technologies to further strengthen GSK's cell therapy pipeline, including GSK3377794, which targets the NY-ESO-1 antigen that is expressed across multiple cancer types.

Lyell's technology is expected to delay the onset of T cell exhaustion, a property that is required for treating solid tumors effectively. Through this, the fitness of T cells could be improved, thereby making the therapy more effective to target solid tumors. Combining GSK's strong cell and gene therapy programs with Lyell's technologies will allow the joint research team to maximize the activity and specificity of cell therapies in solid tumor cancers.

GSK's cell therapy programs will benefit from the collaboration with Lyell's next generation technologies, especially the benefit/

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risk profile of GSK's lead program, GSK3377794 which uses genetically engineered autologous T cells. GSK3377794 is currently in Phase 2 trial on an accelerated development path.

The collaboration will also build on GSK's world-leading manufacturing platform and expertise for cell and gene therapy that delivered the world's first approved *ex vivo* gene therapy (Strimvelis) for ADA-SCID in 2016.

Dr. Rick Klausner, founder and CEO of Lyell Immunopharma commented:

"Our approach is to tackle three of the most significant barriers to T cell efficacy in solid tumors. We are redefining the ways we prepare patient cells to be made into therapies, modulating cells' functionality so that they maintain activity in the tumor microenvironment, and establishing methods of control to achieve specificity and safety for solid tumor-directed cell therapies."

CRISPR AND KSQ THERAPEUTICS JOIN HANDS TO DEVELOP CELL THERAPY PROGRAMS IN ONCOLOGY

Gene editing company CRISPR Therapeutics has announced a license agreement with KSQ Therapeutics to develop their own cell therapy programs in oncology.

Under the terms of the agreement, CRISPR Therapeutics will have access to KSQ intellectual property (IP) for editing certain novel gene targets in its allogeneic oncology cell therapy programs including its allogeneic CAR-T program, and KSQ will gain access to CRISPR Therapeutics' IP for editing novel gene targets identified by KSQ as part of its current and future eTIL™ (engineered tumor infiltrating lymphocyte) cell programs. The financial terms of the agreement were not disclosed.

KSQ Therapeutics uses CRISPR technology to achieve higher probabilities of success in its drug discovery programs. The company is advancing a pipeline of tumor- and immune-focused drug candidates for the treatment of cancer, across multiple drug modalities including targeted therapies, adoptive cell therapies and immunotherapies. KSQ's proprietary CRISPRomics® drug discovery engine enables genome-scale, *in vivo* validated, unbiased drug discovery across broad therapeutic areas.

With headquarters in Switzerland and R&D site in Cambridge, MA, CRISPR Therapeutics is specialized in developing gene-based therapeutics for life-threatening diseases using its proprietary CRISPR/Cas9 gene-editing platform. The company has licensed its CRISPR/Cas9 patent estate for human therapeutic use from its scientific founder, Dr Emmanuelle Charpentier.

Dr David Meeker, CEO of KSQ Therapeutics commented:

"We are thrilled to gain access to CRISPR Therapeutics' foundational IP estate through this agreement. Our eTIL™ programs involve editing gene targets in human TILs that were discovered at KSQ by applying our proprietary CRISPRomics® approach to immune cells in multiple ***in***

vivo models. This agreement clears an important path for us to be able to bring these programs through development and commercialization, leveraging CRISPR Therapeutics' proprietary editing technology."

DICERNA SIGNS DEAL WITH ROCHE TO DEVELOP TREATMENT FOR CHRONIC HEPATITIS B VIRUS INFECTION

Dicerna Pharmaceuticals has entered into a research collaboration and licensing agreement with Roche to develop novel therapies for the treatment of chronic hepatitis B virus (HBV) infection using Dicerna's proprietary GalXC™ RNAi platform technology.

Dicerna Pharmaceuticals is a biopharmaceutical company specialized in developing RNA interference (RNAi) therapeutics. The collaboration will focus on worldwide development and commercialization of DCR-HBVS, Dicerna's investigational therapy in Phase 1 clinical development. The collaboration also includes the discovery and development of therapies targeting multiple additional human and viral genes associated with HBV infection using the technology platforms of both companies.

DCR-HBVS targets HBV messenger RNAs within the hepatitis B surface antigen gene sequence region. Preclinical studies in a mouse model of HBV infection showed 99% reduction in circulating HBsAg.

The company is currently conducting a Phase 1 trial, DCR-HBVS-101, to evaluate the safety and tolerability of DCR-HBVS in normal healthy volunteers and in patients with non-cirrhotic chronic HBV. Dicerna expects to get proof-of-concept data from the trial in the second half of 2019.

The GalXC™ technology is intended to discover and develop next-generation RNAi-based therapies to silence disease-driving genes in the liver.

Under the terms of the agreement, Dicerna will receive $200 million in an initial upfront payment and may be eligible to receive up to an additional $1.47 billion over time for the achievement of specified development, regulatory and commercial milestones. In addition, Dicerna may be eligible to receive royalties based on potential product sales of DCR-HBVS. Dicerna retains an option to co-fund pivotal development of DCR-HBVS worldwide, which if exercised, entitles Dicerna to receive enhanced royalties and co-promote products including DCR-HBVS in the USA.

Dicerna and Roche also agreed to collaborate on the research and development of additional therapies targeting multiple human and viral genes implicated in chronic HBV infection, using technology from both companies, for which Dicerna is eligible to receive additional milestones and royalties on any potential products.

Dr Douglas M Fambrough, Dicerna's CEO commented:

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"Dicerna is excited to collaborate with Roche to realize the full potential of DCR-HBVS and leverage our GalXC platform to target and silence specific genes that contribute to chronic hepatitis B virus infection. With its deep expertise in HBV and established global infrastructure, Roche is ideally suited to help us accelerate the development and commercialization of DCR-HBVS, pursue a cure for chronic HBV infection, and address this serious global threat to public health."

FINANCE

ARCELLX RAISES $85 MILLION IN A SERIES B FINANCING

Arcellx, a privately held biopharmaceutical company, has raised $85 million in an oversubscribed Series B financing. The company will use the funds to advance its differentiated cell therapy platform, Antigen Receptor Complex T cells (ARC-T) + sparX programs, develop a bivalent BCMA-targeted cell therapy for multiple myeloma and a CD123-targeted therapy for acute myeloid leukemia. Proceeds from the fund will also be used to conduct ARC-T + sparX programs for patients with solid tumors and diseases outside oncology.

The finance round was joined by both existing and new investors to Arcellx. New investors Aju IB and Quan Capital co-led the round, followed by Mirae Asset Venture Investment, Mirae Asset Capital, LG Technology Ventures, JVC Investment Partners, and certain funds managed by Clough Capital Partners, L.P. Existing investors Novo Holdings, S.R. One Limited, NEA and Takeda Ventures also participated in the financing.

One of the limitations of current engineered immune cell therapies is that they often target tumors through a mono-specific receptor that is constitutively expressed and active. Arcellx's ARC-T could be readily silenced, activated, and reprogrammed *in vivo* by administration of a tumor-targeting antigen protein called a sparX.

The formation of the ARC-T, sparX, and tumor complex directs the ARC-T to kill the tumor. This therapeutic platform is designed to enhance safety and efficacy while accelerating development by broadening patient accessibility and increasing efficiency of manufacturing relative to existing cell therapies.

As impressive as conventional CAR-T therapies have been, their safety and efficacy profiles are challenged by severe toxicities, high rates of relapse, and challenging

target selection in the solid tumor setting. The ARC-T + sparX platform addresses these concerns by placing ARC-T cells under the control of one or more sparX proteins that uniquely determine how the ARC-T cells recognize tumor, and the speed with which ARC-T cells kill tumor. In the coming months we will begin clinical testing of our lead BCMA-targeted therapy in multiple myeloma.

Arcellx is developing a next-generation T-cell therapy for oncology which is designed to circumvent antigen escape, one of the major limitations of current CAR-T therapies. Instead of engineering T cells to express a CAR, an extracellular binding domain is introduced and the T-cell therapy is administered along with a soluble protein. Once *in vivo* the T cell binds the soluble factor, which in turn binds the cancer cell. The primary benefit of this approach is that if cancer cells lose expression of one of the targets of the soluble factor, clinicians can swap in a different soluble factor for another cancer target. Arcellx's first indication will be BCMA, which is a competitive market, however with the platform differentiation that Arcellx brings to the table it may have an edge.- Mark Curtis

AMARNA THERAPEUTICS RAISES €10 MILLION TO ADVANCE SV40-BASED GENE DELIVERY PLATFORM

Amarna Therapeutics, a privately held biotechnology company developing a next-generation SV40-based gene delivery vector platform named SV*ac*, has announced that it has raised €10 million. The funds will be used to advance development of its SV*ac* platform for a first in man clinical study which is planned in the next 2 to 3 years.

The round was led by Swedish investment company, Flerie Invest AB, and Netherlands Enterprise Agency" (RVO.nl) and the existing shareholder Pim Berger.

In addition to raising new funds, Amarna has also recruited a new Supervisory Board to help support the new phase of its growth and development.

Viral gene delivery vectors that are currently being used for *in vivo* gene therapy have limitations, including instability (lentiviral vectors) and immunogenicity in humans (AAV vectors). Amarna believes that these issues could be overcome by using vectors derived from the macaque polyomavirus Simian Virus 40 (SV40). The company's SV*ac* platform is thought to hold great potential for clinical applications for treating genetic disorders, cancer, allergies and degenerative/inflammatory diseases.

Amarna has genetically engineered the SV40 genome used to produce vector particles and in parallel generated a novel Vero-based packaging cell line named SuperVero that produces similar numbers of vector particles to the currently used packaging cell lines but without contaminating wild

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type SV40 particles. The company claims SV*ac* to be safe, highly efficient, non-immunogenic in humans and vector particles could be cost effectively produced in SuperVero cells.

TMUNITY RAISES $75 MILLION IN SERIES B FINANCING

Investors include venture capital, industry, academia, patient advocacy groups and philanthropy.

Tmunity Therapeutics, in pursuit of its vision to save and improve lives by delivering the full potential of next-generation T-cell immunotherapy, closed a $75 million Series B financing. The financing was led by Andreessen Horowitz (also known as 'a16z'), a venture capital firm that backs bold entrepreneurs building the future through technology and includes participation from a16z's Cultural Leadership Fund. Joining the Series B financing are Westlake Village BioPartners, Gilead Sciences, The University of Pennsylvania, Be The Match BioTherapies and BrightEdge, the philanthropic impact fund of the American Cancer Society.

The proceeds from the Series B will continue to fund ongoing and planned research, clinical development of product candidates, the continued build-out of the Company's proprietary, vertically-integrated viral vector and cell therapy product manufacturing, working capital and other general purposes. Since inception, Tmunity has raised $231 million.

"We are fortunate to be funded by impressive investors who share our commitment to patients and our vision to dramatically change the way cancer is treated," said Usman 'Oz' Azam, MD, President and Chief Executive Officer of Tmunity. "We see ourselves leading the innovation of the future of oncology treatment by uniting our foundational competences in cell therapy with expertise in building new constructs, translating them and getting them into the clinic."

As part of the Series B financing, Jorge Conde, General Partner at a16z, will join the Company's Board of Directors. Mr Conde leads a16z's investments that are at the cross section of biology, computer science and engineering.

"To win the war on cancer, we need smarter weapons. Tmunity's founders Carl June and Bruce Levine invented CAR-T, one of the most profound breakthroughs against cancer in recent history. Together with Oz Azam, who with his team, brought the first CAR-T therapy to market, the company has built a pioneering platform that has produced an unrivaled therapeutic pipeline with programs already in human clinical trials for both solid and liquid tumors. This is the dream team to deliver on the bold and promising mission to cure disease using engineered T-cells," said Conde.

Tmunity's work is focused on the development of T cell-based therapies for the treatment of cancer. The company was founded on a licensing agreement with the University of Pennsylvania.

ARSENALBIO LAUNCHES WITH $85 MILLION SERIES A FINANCING

ArsenalBio, a programmable cell therapy company intending to create highly effective and accessible immune cell therapies was launched last month and is backed up by $85 million Series A financing. The company will integrate technologies such as CRISPR-based genome engineering, scaled and high throughput target identification, synthetic biology, and machine learning to advance a new paradigm to discover and develop immune cell therapies, initially for cancer.

The research which led to ArsenalBio's foundation stems from the contributions of scientific leaders from a consortium of academic medical and research institutions. Investors include Westlake Village BioPartners, the Parker Institute for Cancer Immunotherapy (PICI), Kleiner Perkins, the University of California, San Francisco (UCSF) Foundation Investment Company, Euclidean Capital, and Osage Venture Partners.

Using its programmable and computationally driven approach, the company aspires to develop specialized immune cell therapies with enhanced efficacy, increased patient safety and reduced provider costs.

Unlike the currently developed first-generation T-cell therapies which are designed by inserting a single cell-targeting transgene, a chimeric antigen receptor or a new T-cell receptor through viral delivery, ArsenalBio's approach uses a computationally driven strategy to advance this process by precisely inserting significantly larger DNA payloads without viral vectors, but by using proprietary tools and encoding a broader set of biological 'software' instructions to enable immune cells to effectively target and destroy solid organ and hematologic cancers.

Brook Byers, Founding Partner of Kleiner Perkins of Menlo Park, CA commented:

"ArsenalBio is taking different approaches to gene editing, target selection, cell circuit engineering, and computation to reimagine dosing, delivery, persistence, and affordability of cell therapy. The networks of pharma, science, and talent relationships of PICI, Westlake and Kleiner Perkins is a booster to ArsenalBio's remarkable team and R&D progress".

ADICET BIO RAISES $80 MILLION IN SERIES B FINANCING

Adicet Bio, a pre-clinical stage biopharmaceutical company developing allogeneic cell therapies for cancer using gamma delta T cells, has completed an $80 million Series B financing.

Proceeds from the Series B round will allow Adicet to develop its proprietary technology and advance it into the clinic in Non-Hodgkin's Lymphoma and to advance the solid tumor programs.

New investors include aMoon2 Fund, Regeneron Pharmaceuticals, Inc., Johnson & Johnson

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Innovation – JJDC, Inc. (JJDC), OCI Enterprises, Inc, KB Investment Co., Ltd., Consensus Business Group, SBI JI Innovation Fund, Samsung Venture Investment Corporation, Handok, Inc., and DSC Investment, Inc. All existing investors including OrbiMed, Novartis Venture Fund and Pontifax also participated in the financing.

As part of the Series B financing, aMoon and JJDC will be joining Adicet's Board of Directors. Representing aMoon Fund will be Yair Schindel, MD, Co-Founder & Managing Partner.

RM Global Partners LLC, an investment banking and strategic advisory firm, acted as Adicet Bio's advisors for the Series B financing.

DR MAGALI TAIEL JOINS GENSIGHT BIOLOGICS AS CMO

Gene therapy company GenSight Biologics has appointed Dr Magali Taiel as its new CMO. Dr Taiel replaces Dr Barrett Katz who will continue as a consultant for the company.

Dr Taiel, a medical doctor specialized in Ophthalmology, has had extensive experience both in academic medicine and in the pharmaceutical industry. In her new role, she will oversee clinical development and operations, medical affairs and scientific communication at GenSight Biologics. She will be part of the Executive Committee and will report directly to the CEO Bernard Gilly.

Before joining GenSight, Dr Taiel was VP of Clinical Development at ProQR Therapeutics where she led Clinical Development and Operations to develop antisense oligonucleotides and gene therapy in Inherited Retinal and Neuro-Ophthalmology diseases. Prior to that, she held various international and management positions at Eli Lilly, Pfizer and Servier.

JOHN COX JOINS TORQUE THERAPEUTICS AS CEO

Torque Therapeutics, a clinical stage, product-platform company developing proprietary, first-in-class Deep Primed™ adoptive cell transfer therapeutics for a range of hematologic and solid malignancies, has appointed John Cox as its new CEO.

Mr Cox was most recently CEO of Bioverativ. He led the Bioverativ spin-out from Biogen in 2016 and the sale of Bioverativ to Sanofi for

$11.6 billion in 2018. Prior to that he served in roles of increasing seniority at Biogen, ultimately as EVP of Pharmaceutical Operations and Technology.

Cox became Executive Chairman of Torque in January 2019. During his tenure as Executive Chairman, Torque successfully transitioned from a pre-clinical to a clinical stage company, advanced multiple product candidates in its pipeline, expanded its team with key hires in business development and finance, received Fast Track designation for its lead TRQ-1501 product, and announced a collaboration with Thermo Fisher Scientific to manufacture Torque's Deep Primed™ T Cell immunotherapies, as well as a clinical trial collaboration with Merck to evaluate its lead TRQ-1501 product in combination with Keytruda.

Written by Dr Applonia Rose, Cell and Gene Therapy Insights

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