Mining scientific advice reports on cell-based products; insight in the non-clinical development program.

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Abstract

Aims The field of cell-based therapies for human diseases is currently evolving from promising treatment options to established therapeutic concepts. The design of the non-clinical development program for cell-based products, intended to provide a rationale for treatment and to gain insight into the safety profile, is challenging because of limitations caused by species-specificity. The elements of the non-clinical package for cell-based products were evaluated using advice reports from the European Medicines Agency database from 2013-2018 to identify the approach followed for non-clinical development of these products. Methods The purpose of the in vivo studies was designated to be (a combination of) pharmacology/proof-of-concept, safety, biodistribution and/or tumourigenicity. For biodistribution and tumourigenicity also the need for, type and design of in vitro and in vivo studies were recorded. Results In vivo studies for cell-based therapies were primarily aimed at proof-of-concept (75/86), followed by addressing safety (64/86), biodistribution (49/86) or tumourigenicity (46/86). No animal studies were performed or proposed by sponsors or regulators for six (out of 86) products, which contained cell types that have been studied in humans for a relatively long time. For one-third of the products in vivo biodistribution and/or tumourigenicity studies were not considered necessary. In vivo tumourigenicity studies were regarded of limited value. Conclusions Compared to more conventional medicinal products, the non-clinical development program for cell-based products was more tailored and focussing on proof-of-concept. For tumourigenicity an in vitro approach may suffice. Total omission of in vivo studies appears to be possible for products with sufficient clinical experience.

Introduction

Cell-based therapies are at the forefront of drug innovation. They are highly science-driven, involve innovative drug manufacturing processes and offer potential curative treatment options for diseases, which currently have no or limited treatment options [1]. Hematopoietic stem cell transplantations, as well as adoptive T-cell-therapies and dendritic cell-based therapies, have been used for decades. However, only recently cell-based therapies have evolved from promising treatment options to products for which the therapeutic effect has been established [2,3,4,5]. Since the introduction of the advanced therapy regulation in the EU in 2007 [6] cell-based therapies are regulated as advanced therapy medicinal products (ATMPs) Given the innovative nature of cell-based products and their high variability, development often involves a case-by-case approach [7]. This particularly relates to the non-clinical development program, which is expected to provide a rationale for entering clinical development of a medicinal product and to gain insight into its safety profile. To this end, *in vitro* and *in vivo* animal studies are performed, in which the characteristics of the product and the targeted disease determine the developmental approach to a large extent.

Designing a non-clinical development program for human cell-based products is complex, because species-specificity limits the value of*in vivo* animal studies. Consequently, *in vivo* animal studies with the intended human cell-based product (heterologous model) may not always be relevant. While an animal study with the animal equivalent cells (homologous model) may be informative, some insights into the safety and efficacy of a cell-based product can only be obtained when the product is tested clinically [8,9,10].

While some guidance on the non-clinical development program for cell-based therapies is available in regulatory guidelines [11,12] these are not very prescriptive, as flexibility is needed given the large variability in the types of products. For example, these guidelines state that conventional non-clinical pharmacokinetic studies are not relevant, while animal studies on cell migration (biodistribution) and persistence of the therapeutic cells are generally expected. Presumably, because these data are considered to be of value for the characterisation of the risk profile of the cells and the understanding of their potential therapeutic effect. Still, following the fate of cells *in vivo* is challenging. There are many techniques that can be used to detect specific cells in the body, from basic tissue sampling and subsequent detection of cells by immunohistochemistry (IHC) or specific deoxy nucleic acid (DNA) fragments by polymerase chain reaction (PCR) [13] to highly sophisticated techniques of *in vivo* cell tracking [14]. Nevertheless, they all have their own pro's and con's, and no guidance is provided on which methods are considered most informative.

Regarding the oncogenic potential of cell-based products, the guidelines state that conventional carcinogenicity studies, in which the ability of a medicinal product to transform host cells into tumourigenic cells is evaluated in non-clinical models [15] are not considered appropriate for cell-based therapies [11]. Instead, the potential for tumourigenicity, i.e. tumour formation of the administered (grafted) cells [16] should be considered and addressed as appropriate. Several approaches to study the tumourigenic potential of a cell-based product can be envisioned. These include an *in vivo* assessment of the human cell-based product in animals but also *in vitro*assessment of, for example, genetic stability (e.g. by karyotyping and FISH [17], the presence of transformed or undifferentiated cells in the product (e.g. flow cytometry and qRT-PCR [18], or the ability for anchorage or growth factor-independent growth [19].

To increase the insight in the chosen approach for the non-clinical development for cell-based products we analysed the non-clinical data package of cell-based medicinal products in scientific advice reports from 2013 until 2018 provided by the European Medicines Agency (EMA). In particular, the number and purpose of *in vivo* animal studies was analysed to gain better insight in the prevailing view of sponsors and/or on the relevance of *in vivo* animal data. Furthermore, as biodistribution and the potential for tumourigenicity are most difficult to study in humans, because of the complexity of detection of the administered cell in a patient [20]) and the need for long-term follow up for the assessment of tumourigenic risks [21] we particularly focussed on the need for and type of studies addressing these aspects in the non-clinical development program.

Methods

For the analysis of the non-clinical data packages for cell-based products, we collected scientific advice reports on ATMPs, written between January 2013 and July 2018, from the EMA database. We selected products containing cells (including genetically modified cells) for which the information on the non-clinical development program was available in the advice reports and, if available, from the accompanying briefing books. For all products, it was recorded whether dedicated or combined studies addressing pharmacology, safety, biodistribution and/or tumourigenicity had been performed. In case no studies were performed to address biodistribution and tumourigenicity, it was recorded for what reason: not necessary, planned or whether they were requested by the regulator.

Regarding the biodistribution studies, it was scored per product whether distribution to target and non-target tissues was assessed, whether persistence of the cells was analysed and if quantitative techniques were used. When available, it was noted whether the sponsor regarded the study of value for proof-of-concept and/or safety.

Regarding the tumourigenicity studies, the number and type of studies were recorded. For the *in vitro* studies, the method was scored. For the *in vivo* studies the following aspects on the design of the studies

were noted as well: species, cell dose, administration route and duration.

Analyses of the scored information were performed for all products and for classes of cell-based products when categorisation was applied based on the cell source (autologous versus allogenic) and the presence or absence of genetic modification.

More detail on which data were acquired and how these were analysed is provided in the Appendix.

Results

Non-clinical package

In total 218 ATMP advice reports from 2013 until 2018 were collected from the EMA database, containing information on 153 different products. Of these, 59 were gene therapy medicinal products (GTMPs) not consisting of cells, which were excluded from the study. One product was excluded from the database, because it contained xenogeneic cells and seven products were excluded, because information on the non-clinical program was lacking. The remaining 86 products were categorized based on cell origin and on genetic modification status (Figure 1). The majority of cell therapies in development for which advice was requested during the 2013-2018 period, were not genetically modified (n= 60, 70%). Of these,31 were of autologous origin and 29 were of allogenic origin. The majority of the genetically modified cells were autologous of origin (n=20, 23%) and only six (7%) products were genetically modified, allogenic cells.

In total 234 *in vivo* studies were performed for 80 of the 86 products, and for some products additional animal studies were envisaged. The six products for which *in vivo* animal studies were not conducted, planned or requested did not contain genetically modified cells (data not shown). Pharmacology, which mainly accounts for proof-of-concept (PoC), was studied for most of the products, followed by safety (Figure 2). The number of products with biodistribution and tumourigenicity animal studies was slightly lower. For these types of studies information on biodistribution (8 products) and tumourigenicity (14 products) was not always present in the non-clinical data package provided by the sponsor. It is not known whether sponsors did not regard studies addressing biodistribution and tumourigenicity of value for their product or whether the product was not yet in the stage of development that the issue of biodistribution and/or tumourigenicity study would be relevant to address.

In guidelines for ATMPs it is recommended to combine the various endpoints addressing e.g. pharmacology and safety in one study if possible and sensible, resulting into a so-called combination study [7]. Across the different types of *in vivo* studies, pharmacology was most often evaluated in dedicated studies, whereas safety was often studied in combination studies (Figure 3).

Biodistribution

For at least half of the products the sponsor regarded an *in vivo*animal study of value for the evaluation of the biodistribution of their product, as for 49 of 86 products (57%) a biodistribution study was either conducted (n=40; 47%) or planned (n=9; 10%) (Table 1). For 25 products, the non-clinical data package contained two or more *in vivo* biodistribution studies. For the products (n=37; 43%) for which a biodistribution study was not conducted nor planned, this was agreed upon by the regulators either immediately (n=21) or when supported by further justification (n=3). For five products (6%) the regulators requested a biodistribution study in animals. For eight products (9%), a discussion on the biodistribution was lacking in the sponsor's documentation and was not discussed by the regulators in the advice report. Thus, for 24 of 86 products (28%), both sponsors and regulators considered there was no need for *in vivo* studies on biodistribution. There were no large differences in the presence or absence of *in vivo* biodistribution studies between the product categories (Table 1). Even though for most products biodistribution was studied in a dedicated study (30 of 49 products), sponsors indicated that the results were of value for both understanding the PoC of their product and its safety profile. For several products the value of the biodistribution study for PoC-only or for safety-only was recognized (respectively n=4 and n=11).

For nine products biodistribution was studied in healthy and for twelve products biodistribution was studied

in diseased animals. For nine of these it was explicitly noted that the model was homologous. For three products no information was present on the model and for the remaining 25 products immunocompromised animals, reflecting heterologous models, were used. For 42 products information on the method to evaluate biodistribution was provided. The most commonly used techniques were IHC, quantitative-PCR (qPCR) or reverse-transcriptase PCR (RT-PCR), Fluorescence *In Situ* Hybridization (FISH), and microscopy. For all these techniques tissue samples need to be harvested to allow for detection of the cells. The more sophisticated techniques for studying cell-distribution *in vivo* (such as Positron emission tomography (PET), Single photon emission computed tomography (SPECT), Magnetic resonance imaging (MRI) and Bioluminescence imaging (BLI)) were only used to study biodistribution for three products.

The vast majority of biodistribution studies provided information on the presence of the administered cells in/migration to both target and non-target tissues (40/49; 82% of the products with biodistribution studies, Figure 4). Only for four (autologous cell) products, sponsors considered the option of assessing target tissue only (8% of the studies). For five products it was not mentioned which tissues were assessed (10%). Information on the relative distribution of the administered cells was available for 42 (86%) of the 49 studies, suggesting that the method to determine biodistribution of the therapeutic cells was also (semi-)quantitative.

Information on persistence of the administered cells was available for 44 products (51%). For another 10 products (12%) sponsors planned to evaluate *in vivo* persistence in the future, whereas for 13 products (15%) sponsors did not aim to address it (Figure 5). For the remaining 19 products there was nothing mentioned regarding persistence in the submitted information.

Tumourigenicity

Remarkably, while FDA guidance [11] specifically stipulates that for tumourigenicity studies the intended clinical product should be used (and not analogous animal cells), tumourigenicity was studied in a homologous model for eight products of which three were studied in healthy and five in diseased animals. For one product it was not mentioned and for remaining 37 products immunocompromised animals, reflecting heterologous models, were used. The chosen route of administration (RoA) was most often intravenous or subcutaneous and mainly a dose of $1x10^6$ cells was applied (Table 2). For two products, various doses were used and for ten products the administered dose in the study was not mentioned (most often for planned studies). While there was some variability in the duration of the*in vivo* tumourigenicity studies, a study duration between three and six months was noted for the majority of the products (n=28 of 46).

For most products the tumourigenic potential was addressed by experimental data (*in vitro* and/or *in vivo* studies), only for 10 products (11%) sponsors did not investigate the tumourigenic risk with any experimental data. Of note, for 14 (16%) products there was no information on tumourigenicity studies in the documentation. For nine products (10%), the risk for tumourigenicity was investigated by*in vivo* studies only, for 18 products (20%) the tumourigenic risk was only evaluated by *in vitro* assays, and for 37 products (43%) both *in vivo* and *in vitro* studies were used to address this potential risk (Figure 6). Thus for 46 of the 86 products in the database tumourigenicity was studied and/or planned to be studied*in vivo* (Figure 2). For 18 of the 46 products with *in vivo*tumourigenicity studies, more than two *in vivo* studies tumourigenicity studies were part of the development package. This often concerned the combination of a dose range finding study and a pivotal study, or the assessment of tumourigenic risk upon various routes of administration.

For 16 of the 40 products for which no *in vivo* study was performed or planned, it was explicitly agreed that an *in vivo*study was not necessary, while for six products regulators indicated that the absence of *in vivo* studies could be acceptable provided that the company justified the absence of such a study. This was apparently most often the case for the genetically modified products (Table 3). For four products an *in vivo* animal study was explicitly requested. Products for which tumourigenicity was studied with only *in vitro* studies most often contained genetically modified cells (Figure 6). For products containing allogeneic genetically modified products, tumourigenicity was always discussed

The *in vitro* tumourigenicity studies in the 55 (64%) products most often consisted of karyotyping (n=28) and/or colony formation/proliferation assays (n=22). For 22 products, multiple types of *in vitro* studies

were performed (Table 4).

Discussion

To gain insight into the non-clinical development program for cell-based products we analysed the non-clinical data package of 86 different cell-based products based on scientific advices provided by the EMA between January 2013 and June 2018. A 5-year time period was chosen to ensure that our data contained a sufficient number and diversity of cell-based therapies to allow for a meaningful analysis of the non-clinical development program. In comparison, a previous analysis of the non-clinical program in EMA advices on ATMPs contained 54 ATMPs of which thirteen were somatic cell therapy and eight were tissue engineered products[22].Within the 86 products analysed, a variety of autologous or allogenic cell-based products with and without genetically modifications were included, however the class of products containing genetically modified allogenic cells was only represented by six products.

A non-clinical development program consists of *in vitro* and*in vivo* studies designed to provide a clinical treatment rationale (PoC) and to gain insight into the safety profile of a medicinal product. For cell-based therapies translation of animal data towards the human situation has significant limitations. When testing a human cell-based product in an animal (heterologous model) limitations include possible differences in cell size or cell metabolic rate [23], immunogenicity (xeno-reactions can occur extremely rapid and vigorous) [24] and potential species specificities in cell-cell or cell-environment interactions [25]. These limitations all need to be considered when interpreting study results. A homologous model in which the animal equivalent of the human cell-based product is tested, could be considered as an alternative *in vivo* testing strategy. However, interpretation of results from such a model may be complicated by differences in manufacturing processes of the animal equivalent, deviation of animal response from the human response or absence of an appropriate animal equivalent of the human cells[25].

Interestingly, while the translation of animal data to the human situation has significant limitations for cell-based products, only for six products animal studies were not performed or proposed by the sponsors nor requested by the regulators. These products contained cell types, which have been studied in humans for a relatively long time, such as dendritic cells [26], antigen-specific T-cells [27] and mesenchymal stromal cells (MSC) [28]. Apparently, for these products it was considered that additional animal studies would not provide new insights on these types of products. As the clinical experience with cell-based products is increasing, the knowledge on the (potential) effects of the studied cell types is also increasing. This may result into a reduction or even omission for the need of *in vivo* animal studies for certain types of cell-based therapies.

Still for most products the sponsors considered data from animal studies of value for the development of their product. This was particularly the case for studies on PoC as *in vivo* pharmacology studies were performed for almost all the products. Also safety was studied *in vivo* in animals for the majority of the cell-based products, albeit more often in combination with other endpoints e.g. by also addressing the cellular biodistribution or by including the safety endpoints in pharmacology studies. Thus, it seems that the focus of the non-clinical development program for cell-based products is more on pharmacology/PoC than on safety. This is different from what we see for more 'conventional' medicinal products, where safety is an important component of the non-clinical *in vivo* studies and almost exclusively evaluated in dedicated toxicity studies.

The fact that pharmacology is most often studied in a dedicated study could be explained by a difference in timing of the studies, as non-clinical data are mostly intended to support the rationale for further development before safety studies are performed to ensure the safety of the first clinical trial subjects. Pharmacology studies do not require GLP compliance, in contrast to safety studies. This may have contributed to the higher number of products with pharmacology studies. Notably, although GLP-compliance is formally required for non-clinical safety studies for human medicinal products, for cell-based therapies it has been acknowledged that GLP compliance of *in vivo* safety studies may not always be feasible [29].

In our analysis we particularly focussed on the need for and the type of biodistribution and/or tumourigenicity studies since these aspects are very difficult to study in humans. For more conventional medicinal

products, the kinetics of the product and its safety profile is studied in animals. Interestingly, in our analysis *in vivo* animal studies on biodistribution and/or tumourigenicity were not considered necessary by both sponsors and regulators for approximately one third of the cell-based products irrespective of product type. This observation suggests that for cell-based products a more tailored non-clinical development program is necessary. Thereby sponsors should focus on the need to better characterise the properties of the cell-based product and consider the (im)possibilities of studying a products behaviour in an animal model.

Even though a considerable number of cell-based product in our database are lacking an *in vivo* study on biodistribution, still this type of study was performed for two-thirds of the products and considered to be of value for understanding the PoC and to gain insight into the safety profile. For biodistribution most often a heterologous animal model was used. This is remarkable as cell migration is expected to be dependent on chemotactic signals and cell-cell interactions which might be species-specific and may thus better be studied in a homologous model. Next to distribution to target and non-target tissue, also persistence of the cells was (proposed to be) evaluated for approximately two third of the products, suggesting that sponsors already acknowledge persistence as an important biodistribution endpoint to be investigated. Detection of administered cells in target and non-target tissues was most commonly done by tissue sampling followed by (semi)quantitative detection of these cells with various sensitive techniques. Notably, more sophisticated techniques based on PET, SPECT, and MRI imaging are being developed, which can trace living cells*in vivo* [14,20], allowing for serial sampling and whole-body scanning. However, these techniques were rarely used to study the biodistribution of the products in our database. Possibly these techniques were still not sufficiently developed at the time of the design of the non-clinical studies for our analysed products. It is also possible that these techniques were not (yet) attractive due to high costs, limited access and/or the perception that these techniques are too novel and have not yet proven their value to ensure their acceptability by regulators. Notably, these newer techniques of cell tracking may also become suitable for the evaluation of biodistribution of cellular therapeutics in humans, thus in the clinical setting. Clinical biodistribution studies would be of much more relevance as the therapeutic product can be tested directly in the target 'species'. The hope is that these more advanced methods analysing distribution of cellular therapies, will be implemented in due time and that they could possibly obviate the need for extensive evaluation of *in vivo*biodistribution of human cell-based products in animals.

As tumourigenicity is a concern associated with cell-based products, it is not surprising that for most products experimental data was used to address the potential for tumourigenicity. For half of the products this was planned or performed to be addressed by means of *in vivo*animal studies. For four-fifths of these products, *in vitro*studies supplemented the tumourigenicity evaluation. One-fifth thus leaned on *in vivo* studies only. The design of these *in vivo* studies appeared rather similar across the various products, and all tended to follow the WHO guidance [16] on the assessment of tumourigenicity of mammalian cells (i.e. $1x10^6$ cells, subcutaneous administration, 3 to6 months in duration), except that intravenous administration was often used as well to better reflect the clinical RoA. Interestingly, this WHO guidance was developed for characterisation of a master or a working cell bank (MCB, WCB) [16], and not to address the tumourigenic potential of human cell-based products. The suitability of this study design for evaluation of the tumorigenic risk of cell-based products has not been established. Recently, the added value of *in vivo* tumourigenicity studies has been questioned [21]. With respect to this, it is interesting that only for a minority of products the *in vivo* study was considered to have some relevance by the regulators or experts. Despite the limited relevance, the non-clinical data package on the tumourigenic potential was considered sufficiently informative for most of the products. This implies that while limited value was given to the *in vivo*studies, the value of *in vitro* studies for the assessment of tumourigenicity is recognized. Notably, an *in vitro* -only evaluation of tumourigenic risk was accepted for two types of products, i.e. for autologous cells and for genetically modified products. For the genetically modified products *in vitro* evaluation of insertion site analyses (ISA) seem to be sufficient for addressing the tumourigenic risk. Possibly, the need for *in vivo* animal studies for the safety assessment of cell-based products may be further reduced and more sensitive and standardised *in vitro* assays may be developed to characterise the risk for tumourigenicity [18,21,30].

To conclude, our analysis has given insight into the non-clinical development program for cell-based products,

specifically on the number and types of studies performed for the various types of products. It appears not possible to define a common route to-be-followed for the non-clinical development program of cell-based therapies. While it is clear that during development studies on the pharmacology, biodistribution, general safety and tumourigenicity should be considered, it is not evident that for all these aspects animal studies need to be performed or how they should be designed. Not only because the variety of products prohibits a-one-size–fits-all approach for non-clinical development, but also because insights are changing and clinical experience is growing, which can affect the need for animal studies for future products. Moreover, technical possibilities to study certain aspects may change in time as well. Therefore, the recommendation remains to tailor the non-clinical development program for cell-based products to the specific need for information, depending on the characteristics of the product and take into consideration available knowledge and relevance of *in vivo* animal studies. To this end, early dialogue between sponsors and regulators will remain utterly valuable.

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Tables

Table 1: Availability of biodistribution studies in the non-clinical development

	Performed	Planned	Requested	Absence to be justified	Not necessary
Autologous not genetically modified (n=31)	11	3	3	0	10
Allogeneic not genetically modified (n=29)	17	3	0	2	6
Autologous genetically modified (n=20)	9	3	1	0	4
Allogeneic genetically modified (n=6)	3	0	1	1	1
Total of biodistribution studies	**40**	**9**	**5**	**3**	**21**

Table 2: Design of the *in vivo* tumourigenicity studies, regarding applied dose, route of administration and duration

	Dose	Dose	Dose	Dose	Dose	Route of administration	Route o
	10e5	**10e6**	**10e7**	**various**	**n.m.**	**S.C.**	**I.V.**
Autologous not genetically modified (n=17)	3	6	2	0	6	5	3
Allogeneic not genetically modified (n=19)	1	11	5	1	2	11	6
Autologous genetically modified (n=12)	5	5	0	0	2	1	4
Allogeneic genetically modified (n=3)	1	0	1	1	0	1	0
Total46	10	22	8	2	10	18	13

n.m. = not mentioned, I.V. = intravenously, S.C.= subcutaneously

Table 3: Availability of *In vivo* studies for tumourigenicity in the non-clinical development plan

	Performed	Planned	Requested	Absence to be justified	Not necessary
Autologous not genetically modified (n=31)	11	5	2	1	6
Allogeneic not genetically modified (n=29)	14	4	0	1	5
Autologous genetically modified (n=20)	8	1	1	3	4
Allogeneic genetically modified (n=6)	3	0	1	1	1

	Performed	Planned	Requested	Absence to be justified	Not necessary
Total of tumourigenicity studies	**36**	**10**	**4**	**6**	**16**

Table 4: Overview of the types of*in vitro* tumourigenicity studies performed within the four product categories

	Karyotyping	Colony formation	Genomic analysis	Immortali-zation	Ot
Autologous not genetically modified (n=13)	9 (69%)	9 (69%)	4 (31%)	2 (15%)	2 (
Allogeneic not genetically modified (n=21)	14 (67%)	10 (48%)	5 (24%)	4 (19%)	3(
Autologous genetically modified (n=16)	2 (13%)	3 (19%)	2 (13%)	10 (63%)	1 (
Allogeneic genetically modified (n=5)	3 (60%)	0 (0%)	3 (60%)	0 (0%)	0 (
Total (n=55)	28 (52%)	22 (40%)	14(25%)	16 (29%)	6 (

Of note; the number of specific in vitro analysis used per product category was calculated as a percentage of the total products in that category

Figure legends

Figure 1: Overview of selection process of scientific advices. All advice reports from the EMA from 2013 -2018 were collected and the advice reports on products containing human cells for which non-clinical data information was present in the reports were selected. ATMP: advanced therapy medicinal products.

Figure 2: Type of studies performed for the various product classes. In total 234 *in vivo*studies were performed for 86 products. The purpose of the studies is shown for the products that are categorized based on cell origin and genetic modification status that is depicted by the different colours.

Figure 3: Percentage of dedicated and combination studies. The number of *in vivo* animal studies is related to the total amount of products (86) addressing pharmacology (75), safety (64), biodistribution (49) and tumourigenicity (46).

Figure 4: Migration analysis in biodistribution studies. The migration towards target tissue only, or both to target and non-target tissues was recorded for the biodistribution studies and specified for product categories based on their cell origin and genetically modification status.

Figure 5: Analysis of the persistence of the cells in the biodistribution studies. The persistence of the cells in the animal was either evaluated (Yes), not evaluated (No) or planned (Planned) to be evaluated. For some products persistence of cells was not mentioned in the advice reports (Not mentioned).

Figure 6: Approach for estimation of the tumorigenic risk. The approach for evaluation of the tumourigenic risk by *in vivo* and/or *in vitro* studies or none of these (‘*In vivo’, ‘In vitro’* , or both *‘in vivo & in vitro’,* or ‘none’). For some products, the tumourigenic risk is not addressed in the scientific advice (‘Not mentioned’).

Appendix

Method for processing and analysing of scientific advice reports

A scoring table and a partly overlapping analysis table were designed to record and analyse the information on the non-clinical development program as present in scientific advice reports and corresponding provided documentation from the sponsor. Both tables consisted for three parts 1) general data and information regarding *in vivo* animal studies, 2) biodistribution studies and 3) tumourigenicity studies. For a coded descriptive the options are given in between brackets. For an open descriptive this is listed as ‘specified’ in between the brackets.

Scoring table

Part 1: Elements scored regarding general and in vivo data

Advice number; Product name; Indication; Product type; Cell (and vector) type; First and second advice coordinator; Topic(s) of question(s); Occurrence of discussion meeting; *in vivo* studies (no, yes, not mentioned); Goal of *in vivo* studies (Proof of Concept (PoC), Biodistribution, Tumourigenicity, Safety).

Part 2: Elements scored regarding biodistribution studies

Availability *in vivo* study (no, yes); Reason for absence of the study (not necessary, planned, requested, not necessary but justification required, not mentioned); Analysis (clinical, non-clinical) Species used (specified); Animal model used (healthy, disease model, immune-compromised); Dose used (specified); Method for labelling and detection (specified); Sampling time points (specified); Site of Administration (specified); Migration (target tissues, both target and non-target tissues); Quantifiable method (no, yes, not mentioned); Persistence studied (no, yes, planned, not mentioned); Informative (no, yes); Informative for (PoC/Mechanism of Action (MoA), Safety, both)

Part 3: Elements scored regarding tumourigenicity studies

Availability *in vivo* study (no, yes); Reason for absence of the study (not necessary, planned, requested, not necessary but justification required, not mentioned); Species used (specified); Animal model used (healthy, disease model, immune-compromised); Survival product in animal (no, yes, not mentioned); Control group (no, yes, not mentioned); Sample size (specified); Cell dose (specified); Route of Administration (RoA, specified); Duration of the study (specified); Tumor formation (no, yes of human origin, yes of animal origin, unknown);); Relevance (no, yes); Available *In vitro* studies (no, yes, requested, not mentioned); Method *in vitro* study (specified); *In vivo* and *In vitro* present (only *in vivo,* only *in vitro* , both, neither performed, not mentioned); Literature on cell type/cell product (no, yes, requested); Informativeness total package (no, partially, yes, not discussed), Reason for regarded informative (specified)

Analysis table

Part 1: purpose of in vivo animal studies

PoC/ MoA; Biodistribution; Tumourigenicity; Safety; Biodistribution & safety; Biodistribution & POC; Tumourigenicity & Safety; Biodistribution & tumourigenicity & safety; Biodistribution & PoC & safety; Bio & POC & safety; POC & safety; POC & tumourigenicity & safety; Biodistribution & tumourigenicity

Part 2: need for, type and design of in vivo biodistribution studies

Available *in vivo* study, Reason for absence (not necessary, planned, requested, not necessary but justification required, not mentioned); Multiple studies performed (no, yes); Homologous model (not (known), yes, both homologous and heterologous); Migration (target tissues, both target and non-target tissues); Quantifiable method (no, yes, not mentioned); Persistence studied (no, yes, planned, not mentioned); Informative (no, yes); Informative for (PoC/MoA, Safety, both)

Part 3: need for, type and design of invitro and in vivo tumourigenicity studies

Available *in vivo* study, Reason for absence (not necessary, planned, requested, not necessary but justification required, not mentioned); Homologous model (not (known), yes, both homologous and heterologous); Dose of 10ˆ5; Dose of 10ˆ6; Dose of 10ˆ7, RoA subcutaneous, RoA other (specified); Duration 0-3 months; Duration 3-6 months; Duration 6+ months; Presence Integration site analysis; Tumor formation (no, yes of human origin, yes of animal origin, unknown); Relevance (no, yes); Available *In vitro* studies (no, yes, requested, not mentioned); Karyotyping; Colony formation/Proliferation; Genomic analysis (transcriptional/epigenetic/SNP/microarray, telomere length); Immortalization (IVIM, senescence, apoptosis); Other (specified); *In vivo* and *In vitro* present (only *in vivo,* only *in vitro* , both, neither performed, not mentioned); Informativeness total package (no, partially, yes, not discussed), Reason for regarded informative (specified)