First-in-human Phase 1 CRISPR Gene Editing Cancer Trials: Are We Ready?

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Abstract: A prospective first-in-human Phase 1 CRISPR gene editing trial in the United States for patients with melanoma, synovial sarcoma, and multiple myeloma offers hope that gene editing tools may usefully treat human disease. An overarching ethical challenge with first-in-human Phase 1 clinical trials, however, is knowing when it is ethically acceptable to initiate such trials on the basis of safety and efficacy data obtained from pre-clinical studies. If the pre-clinical studies that inform trial design are themselves poorly designed – as a result of which the quality of pre-clinical evidence is deficient – then the ethical requirement of scientific validity for clinical research may not be satisfied. In turn, this could mean that the Phase 1 clinical trial will be unsafe and that trial participants will be exposed to risk for no potential benefit. To assist sponsors, researchers, clinical investigators and reviewers in deciding when it is ethically acceptable to initiate first-in-human Phase 1 CRISPR gene editing clinical trials, structured processes have been developed to assess and minimize translational distance between pre-clinical and clinical research. These processes draw attention to various features of internal validity, construct validity, and external validity. As well, the credibility of supporting evidence is to be critically assessed with particular attention to optimism bias, financial conflicts of interest and publication bias. We critically examine the pre-clinical evidence used to justify the first-in-human Phase 1 CRISPR gene editing cancer trial in the United States using these tools.

We conclude that the proposed trial cannot satisfy the ethical requirement of scientific validity because the supporting pre-clinical evidence used to inform trial design is deficient.

Keywords: CRISPR, Phase 1, Cancer, Gene editing, Research ethics, Scientific validity.

1. INTRODUCTION

Select cancer patients in China and in the United States, who are in relapse or have treatment-refractory tumors, are now eligible to participate in first-in-human Phase 1 CRISPR gene editing cancer trials. The trial in China (currently underway) is for patients with stage IV metastatic non-small cell lung cancer [1]. The prospective trial in the United States is for patients with melanoma, synovial sarcoma, and multiple myeloma [2]. For some, these are exciting times given the potential of gene editing tools to treat human disease. Others, however, are somewhat more reserved in their enthusiasm. Among the skeptics are those who wonder whether CRISPR gene editing cancer trials are premature – likely to be proven unsafe, and to have put trial participants at risk for no potential benefit.

In this article, we briefly describe the proposed first-in-human Phase 1 CRISPR gene editing trial for cancer in the United States. This trial was reviewed in June 2016 by the Recombinant DNA Advisory Committee (RAC) at the United States National Institutes of Health (NIH). The RAC is an advisory committee tasked with providing the NIH Director with advice and recommendations. Any proposed gene editing research that falls within the scope of the NIH guidelines is subject to RAC review. Next steps before research participants can be enrolled include review and approval from the United States Food and Drug Administration (FDA), and from the University of Pennsylvania Institutional Biosafety Committee (IBC), Conflict of Interest Standing Committee, and Institutional Review Board (IRB).

Following this description of the proposed clinical trial, we critically evaluate the quality of the pre-clinical evidence presented to the RAC in support of the submission to initiate clinical research. We highlight features of the pre-clinical...
evidence that, in our estimation, threaten the ethical requirement of scientific validity. Ezekiel Emanuel and colleagues David Wendler and Christine Grady suggest that scientifically valid research “must have a clear scientific objective; be designed using accepted principles, methods and reliable practices; have sufficient power to definitively test the objective; and offer a plausible data analysis plan” [3]. In more colloquial terms, Kirstin Borgerson writes:

“What the ethical requirement of scientific validity is meant to get at is that the science in question is, to put it simply, good science. It follows the norms of science appropriate in the particular discipline. It models good scientific inquiry. It is well-designed [to advance knowledge].” [4: p.394].

We conclude that the one pre-clinical study in mice used to justify the first-in-human Phase 1 CRISPR gene editing cancer trial in the United States does not satisfy the ethical requirement of scientific validity. Moreover, the translational distance between the pre-clinical study and the proposed clinical trial is unnecessarily wide – the quality of pre-clinical evidence is seriously deficient. As a direct consequences of this, the ethical requirement of scientific validity for clinical research may not be satisfied. This conclusion suggests that there is reason to question current enthusiasm for proceeding with human somatic cell gene editing using existing national regulatory frameworks and oversight mechanisms (e.g. laws, regulations, policies, guidelines, standards, and professional norms and practices).

2. U.S. FIRST-IN-HUMAN PHASE 1 CRISPR GENE EDITING CANCER TRIAL

In 2016, investigators at the MD Anderson Cancer Center in Texas, the University of California in San Francisco and the University of Pennsylvania in collaboration with the Parker Institute for Cancer Immunotherapy announced plans to proceed with a CRISPR gene editing trial using autologous T cells entitled “Phase I Trial of Autologous T Cells Engineered to Express NY-ESO-1 TCR and Gene Edited to Eliminate Endogenous TCR and PD-1”. The usual purpose of a Phase 1 clinical trial is to assess the safety and dosage of a novel intervention [5, 6]. Some Phase 1 trials, however, also seek preliminary evidence of efficacy, [7] and this is mostly the case with cancer trials.

The proposed Phase 1 clinical trial uses a competitive repopulation strategy that is basket designed to test several endpoints in eighteen (18) research participants with refractory tumors, including melanoma (n=6), synovial sarcoma (n=6) and multiple myeloma (n=6), for whom there are no effective therapies. The primary endpoints for this first-in-human combination of immunotherapy and gene editing are patient safety and feasibility, as well as manufacturing feasibility. The secondary endpoints are a clinical assessment of anti-tumor responses and survival, as well as an examination of T cell bioactivity, immunogenicity, engraftment, persistence, and trafficking. The investigators acknowledge (in the consent documents) that the research participants may not get any personal medical benefit from participating in this Phase 1 clinical trial.

The basket design consists of an open label pilot study where peripheral blood lymphocytes will be collected from research participants. The T cells, which are a sub-type of lymphocyte in peripheral blood involved in cell-mediated immunity (i.e. the immune response), will then undergo transduction using a lentiviral vector to express a new high affinity T cell receptor with specificity for the NY-ESO-1 peptide (NY-ESO-1 TCR). NY-ESO-1 is a highly immunogenic antigen expressed on human tumors. For example, NY-ESO-1 is expressed on melanoma, synovial and myxoid sarcoma and advanced myeloma tumors at a rate of 28-45%, greater than 70% and approximately 50% respectively [2]. In addition to allowing the transduced T cells to target the NY-ESO-1 peptide expressed on human tumors, engineering NY-ESO-1 TCR aims to overcome an incapacity to isolate and propagate large numbers of T cells with a defined specificity and phenotype [8].

The T cells will also be gene edited using CRISPR technology to knock-out the gene loci for the α and β chains of the endogenous T cell receptor (TCR α and TCR β respectively) as well as the Programmed Cell Death Protein-1 (PD-1). The rationale for gene editing TCR α and TCR β is to: (1) reduce TCR mispairing with NY-ESO-1 TCR and the possible formation of neo-reactivity [see, e.g. 8, 9]; (2) reduce the possibility of autoimmunity; as well as (3) promote the expression of exogenous NY-ESO-1 TCR. It is hypothesized that gene editing PD-1 will prevent exhaustion and therefore maintain T cell activity in the presence of the checkpoint ligands, PD-L1 and PD-L2, including those expressed by tumor cells or with cells within the tumor micro-environment [see, e.g. 10, 11].

Following transduction and gene editing, the T cells – which are known as NY-ESO-1 Redirected CRISPR Edited T Cells – will be expanded ex vivo. Meanwhile, the trial participants will undergo lympho-depleting chemotherapy, and thereafter will receive a single dose of 1x10⁸ autologous NY-ESO-1 Redirected CRISPR Edited T Cells/kg. The current investigators (as well as other investigators) have used T cells expressing NY-ESO-1 TCR in other pre-clinical studies and in human clinical trials and have shown safety and efficacy [2, 10; see, e.g., 12, 13]. The current trial is novel, ¹

¹ A lentivirus is a virus-like particle that can be used as a vector in both clinical and non-clinical research to deliver recombinant DNA molecules known as transgenes into host cells.

² The data from this Phase I/II clinical trial indicated that although T cells remained functional in some research participants for up to a year after infusion, there was exhaustion over time [11].

³ According to Lo and colleagues [6], the aims of Phase 1 clinical trials are “to assess the safety and feasibility of the investigational intervention and to determine dosages for subsequent clinical trials. Direct therapeutic benefit, although hoped for, is unlikely in early trials, particularly if the first participants receive low doses.”
however, as it includes the creation of NY-ESO-1 TCR in conjunction with the use of CRISPR technology to triple edit TCR α, TCR β and PD-1 genes. The investigators believe that autologous NY-ESO-1 Redirected CRISPR Edited T Cells will have improved antitumor activity and enhanced persistence [e.g., 2, 13].

3. VALIDITY AND PRE-CLINICAL EVIDENCE

An overarching ethical challenge with first-in-human Phase 1 clinical trials is knowing when it is ethically acceptable to initiate such trials. This is not a move to be made on the basis of intuition, institutional pressure, career advancement, prospective financial rewards, anticipated accolades, or international dueling. A key resource used to justify this move is safety and efficacy data from pre-clinical studies.

In *Gene Transfer and the Ethics of First-in-Human Research: Lost in Translation* [14], Jonathan Kimmelman developed a four-part framework to assist investigators and reviewers in deciding when it would be ethically acceptable to initiate first-in-human clinical trials. The framework aimed to ensure modest translational distance (i.e., a narrow inferential gap) between pre-clinical and clinical research. This 2010 framework called for an assessment of the internal and external validity of pre-clinical studies used to support Phase 1 clinical trials, as well as an assessment of the correspondence between the experimental design of the pre-clinical studies and subsequent clinical trials. It also called for a critical appraisal of the credibility of the supporting evidence with particular attention to optimism bias, financial conflicts of interest and publication bias.

More recently, in 2014, following a systematic review of guidelines for the design and execution of in vivo animal studies [15], the original framework has been refined in collaboration with Valerie Henderson. Together Henderson and Kimmelman propose a structured process for evaluating pre-clinical evidence in terms of potential threats to internal validity, construct validity, and external validity [16]. Below, we evaluate the validity and the credibility of the pre-clinical evidence presented in support of the proposed first-in-human Phase 1 CRISPR gene editing cancer trial in the United States.

3.1. Internal Validity

Kimmelman defines internal validity as “the ability to make causal inferences from an experimental result” [14: p.119]. Of concern with pre-clinical studies as supporting evidence for clinical studies are the risks of “biases or random errors that lead to spurious causal inferences” [16: p.51]. Minimizing these risks requires close attention to various elements of trial design such as sample size (a-priori power calculations), randomized allocation of animals to treatment, blinding of outcome assessment, dose-response relationships and selection of appropriate controls groups. According to Kimmelman, frequently little attention is paid to these elements at the pre-clinical stage [e.g. 14: pp.110-131].

Our review of the (only) one pre-clinical gene editing study using NY-ESO-1 Redirected CRISPR Edited T Cells in mice presented to the RAC suggests problems with these methodological elements. For a start, the sample size is surprisingly small. There were only three treatment groups for a total of 17 animals. Moreover, there were only five animals in the treatment group using NY-ESO-1 Redirected CRISPR T cells. In addition, there appears to have been no randomized allocation, no blinding of outcome assessment, no T cell infusin dose response assessment, and no robust statistical analysis. On the assumption that such information would have been provided to the RAC, if available, the apparent absence of such data is concerning and arguably represents a significant lacuna with respect to internal validity. Furthermore, although the investigators disclosed that NY-ESO-1 Redirected CRISPR Edited T Cells could be maintained for at least 2 months, they failed to assign a group of control tumor negative animals with an infusion of NY-ESO-1 Redirected CRISPR Edited T Cells to evaluate adverse effects over a longer period of time.

3.2. Construct Validity

According to Henderson and Kimmelman, construct validity concerns the “relationship between behavioral outcomes in animal experiments and human behaviors they are intended to model” [15: p.2]. Construct validity also extends to the relationship between animals and humans, as regards the underlying condition, treatments, causal pathways, experimental operations and clinical scenarios. At issue is whether the animal model(s), the interventions, and outcome assessments in pre-clinical studies are good representations of the human condition under study [17].

Construct validity may be threatened if investigators “err in executing experimental operations” [15: p.2] or when “physiological derangements driving human disease are not present in the animal model” [15: p.2]. Moreover, construct validity is further threatened when there is no interpolation between data from published (potentially innumerable pre-clinical or clinical) research and first-in-human trials, or when investigators rely on pre-clinical studies that do not validate the hypothesis being tested [e.g. 14: pp.110-131]. Henderson and Kimmelman state that threats to construct validity “are reduced by articulating, addressing, and confirming theoretical presuppositions underlying clinical generalization” [15: p.2].

3.2.1. Choice of Tumor Cell Line

A first threat to construct validity relates to the cancer introduced into the mouse model. The investigators introduced a human lung cancer cell line, A549-ESO-CBG (which was...
HLA-A2+ and NY-ESO-1+) into genetically modified NOD Scid Gamma (NSG) mice. However, the proposed Phase 1 clinical trial does not plan to recruit lung cancer patients. The target population includes patients with melanoma, synovial sarcoma or multiple myeloma.

The four cancer subtypes have different molecular and phenotypic characteristics. Arguably the investigators should have introduced a melanoma, plasma or a sarcomatous cell line expressing HLA-A2+ and NY-ESO-1+ into the mouse model. Alternatively, instead of using long-established cell lines (which have their own limitations), they could have used biopsied cells from cancer patients with melanoma, synovial sarcoma, or multiple myeloma. For even greater construct validity, the investigators could have introduced biopsied cells from prospective research participants into the mouse model. Cells from a biopsy would more accurately model the biology of the original tumor [18].

To be sure, there are potential shortfalls with the use of patient-derived biopsied cells, including the need for immunodeficient hosts, the time required to grow the tumor in the animal model, the loss of non-transformed stromal elements, as well as the cells not exactly resembling the human disease due to the possible process of selection pressure that may change the clonal composition of the engrafting tumor to more malignant cells and clones [e.g. 18-22]. These limitations, however, apply equally to established cell lines.

Patient-derived biopsied cells have been shown to be phenotypically stable across multiple transplant generations and typically retain the principal histological, transcriptomic, proteomic and genomic characteristics of their donor tumor while showing comparable treatment responses to those observed clinically [e.g. 18, 22-28]. For example, histological features including gland formation and keratin deposition have been shown to be comparable to the original tumor, while the gene expression profile of patient-derived cells also clusters with the original tumor [e.g. 18, 29, 30]. These characteristics suggest that the treatment regimen in the pre-clinical study would be more comparable to the human clinical trial if biopsied patient-derived cells were used in pre-clinical studies instead of established cell lines [e.g. 23, 31, 32].

No robust causal inferences for the first-in-human Phase 1 CRISPR gene editing trial in patients with melanoma, synovial sarcoma or multiple myeloma can be made on the basis of one pre-clinical study in mice with a human lung cancer cell line. The risk of mischaracterizing the anti-tumor response of NY-ESO-1 Redirected CRISPR Edited T Cells on a human lung cancer cell line is considerable.

3.2.2. Anatomical Location of Cancer, Co-Interventions, Sex and Age

If investigators deliver a cancer cell line in a pre-clinical study to an anatomical location in an animal that is different to the tumor location in humans, then construct validity is threatened [14: pp.110-131]. In the one pre-clinical study presented to the RAC, NSG mice were injected with a human lung cancer cell line in the right flank. Information about the location of tumors in research participants was not disclosed. It is likely, however, that the right flank is a different anatomical location to the three sub-types of cancer in the proposed Phase 1 clinical trial.

For example, melanoma is usually located on the skin but has a high propensity to metastasize to the brain [33, 34]. Given that the target population includes patients with melanoma, the pre-clinical study should have investigated whether the NY-ESO-1 Redirected CRISPR Edited T Cells could migrate across the blood-brain barrier. The investigators included no statements regarding the potential ability of NY-ESO-1 Redirected CRISPR Edited T Cells to cross the blood-brain barrier in the proposed Phase 1 clinical trial. This is an important consideration in the event that the melanoma has brain metastasized in the research participants. This speaks to the value of having more than one pre-clinical experiment with cancers in anatomical locations that better mimic the disease under study.

Also relevant is the fact that trial participants will undergo chemotherapy prior to the infusion of NY-ESO-1 Redirected CRISPR Edited T Cells. There is no equivalent intervention in the pre-clinical study [18]. While it is true that NSG mice lack a complete functional immune system so their inflammatory immune cell response is deficient, it is possible that the chemotherapy (an active drug) is a confounding factor and thus should have been controlled for in pre-clinical studies. As such, the NSG mice were not matched to co-interventions. Moreover, as NSG mice lack mature T cells, B cells, natural killer cells and are deficient in multiple cytokine signalling pathways, the pre-clinical study cannot assess the possibility of cytokine release syndrome [35]. Cytokine release syndrome is a life-threatening complication that can cause brain edema, neurological damage and death. This has been shown to occur in clinical trials using anti-CD19 CAR-T cell therapies [36, 37].

Finally, the investigators did not design the pre-clinical study using mice from both sexes to match the animal model to the sex of patients in clinical setting, nor did they use different age groups.

No robust causal inferences for the first-in-human Phase 1 CRISPR gene editing cancer trial can be made on the basis of one pre-clinical study in mice with a cancer in an anatomical location different from where the cancer is likely to be in a human population. Additional confounding factors include the absence of an equivalent to chemotherapy in the pre-clinical study as well as a failure to study both sexes in a range of ages. As such, the risk of mischaracterizing the efficacy of NY-ESO-1 Redirected CRISPR Edited T Cells on a tumor is considerable.

3.2.3. Choice of Donor T Cell Source

Another issue with construct validity relates to the reliance on data from in vitro experiments using T cells from two healthy donors to confirm the efficacy of lentiviral transduction and gene editing. The problem with this strategy is twofold, (1) an exceedingly small cohort (two cell sources) and (2) the exclusive use of T cells from healthy donors. Thereafter, the investigators used the same T cells in the one pre-clinical study in mice.

The investigators recognize the limitation of using T cells from a small cohort to determine the efficacy of lentiviral
transduction and gene editing. They have plans to expand the *in vitro* analysis using cells from a greater number of healthy donors in the future. This should have been done prior to the RAC submission, however, as information from additional cell sources could have usefully informed clinical trial design.

The investigators should also have tested the efficacy of lentiviral transduction and gene editing in T cells from individuals with cancer, including patients with melanoma, synovial sarcoma, or multiple myeloma, and thereafter used the same T cells in pre-clinical animal studies to determine the two study endpoints of (1) tumor size and (2) percent survival. Information about the efficacy of lentiviral transduction and gene editing in cancer patients would have been a prudent step prior to the RAC submission. Although the route/method of treatment delivery (*i.e.* infusion) in the pre-clinical and clinical research appear to match, as do the two study endpoints (*i.e.* clinical assessments of anti-tumor responses and survival), the use of cancer patient derived T cells in the pre-clinical study in mice (and subsequently in larger animal models that more closely mimic the human disease, such as canines) would have improved construct validity between the pre-clinical and clinical research.

No robust causal inferences for the first-in-human Phase 1 CRISPR gene editing cancer trial can be made on the basis of the pre-clinical data obtained using T cells from only two donors that were not sourced from a cancer patient population. The risk of mischaracterizing the efficacy of T cells from healthy donors is considerable.

### 3.2.4. Heterogeneous Transduced and Gene Edited Cell Populations

The proposed Phase 1 CRISPR gene editing cancer trial may create as many as 16 populations of autologous NY-ESO-1 Redirected CRISPR Edited T Cells. This is because of the possible permutations and combinations resulting from the plan to first introduce a lentiviral vector to express NY-ESO-1 TCR and second to delete the gene loci for the TCR α, TCR β and PD-1.

At one extreme, there could be wild-type endogenous T cells that have not been successfully modified with the lentiviral vector and CRISPR technology. This cell population likely would be safe but not efficacious. At the other extreme, there could be T cells expressing NY-ESO-1 TCR with endogenous TCR α, TCR β and PD-1 disruptions. This successfully modified cell population presumably would have enhanced anti-tumor effects and be less susceptible to exhaustion by PD-L1 and PD-L2. In between these two phenotypic extremes there would be various cell populations. To date, limited available data indicate that 49% and 62% (respectively) of cells from the two healthy donors expressed NY-ESO-1 TCR after lentiviral transduction. However, a significant proportion (approximately 43%) of all the transduced cells, were not PD-1 edited. Despite evidence of significantly varying levels of genetic modification (transduction and gene editing), there is no plan to screen for a specific cell population prior to infusion into research participants.

In the event of any observed effect in the one pre-clinical study in mice, no robust causal inferences for the first-in-human Phase 1 CRISPR gene editing cancer trial could be made about the safety and efficacy of any research intervention given the potential heterogeneity of the cell populations.

### 3.2.5. Summary of Concerns Regarding Construct Validity

In our view, construct validity is threatened as the pre-clinical study did not validate the hypothesis that is to be tested in the Phase 1 clinical trial.

Firstly, the investigators should have tested the efficacy of lentiviral transduction and gene editing in more than two sources, as well as used T cells from cancer patients (including perhaps prospective research participants) with melanoma, synovial sarcoma, or multiple myeloma. There should have been toxicology and efficacy studies of the various investigational cell populations to address the limitations with respect to the potential heterogeneity of the cell populations.

In the event of any observed effect in the pre-clinical research, no robust causal inferences can be made about the safety and efficacy of any research intervention given the potential heterogeneity of the cell populations. As well, there is failure to take into account a range of potential confounding factors including co-interventions, sex and age.

In the event of any observed effect in the pre-clinical research, no robust causal inferences can be made about the safety and efficacy of any research intervention given the potential heterogeneity of the cell populations.

### 3.3. External Validity

Kimmelman describes external validity with reference to the importance of “conducting replication studies that vary experimental conditions” [14: p.120] in order to effectively test whether (and if so, to what extent) “cause and effect relationships hold up under varied conditions.” [17]. Among the many possible confounders are the replication of different models of the same disease, independent replication, replication of different species, standardization of methods, and
reproducibility of results [38]. More recently Henderson and Kimmelman have suggested that external validity threats can be tempered by ensuring that the pre-clinical research is conducted in “(1) more than one model, (2) more than one laboratory and (3) more than one species” [16: p.51].

Now, some commentators argue that non-human animal studies, as currently conducted, cannot reasonably predict the outcome of human trials [39]. This is because non-human animals are poor models for the majority of human diseases [see, e.g. 40, 41] due to genetic, molecular, physiological, immunologic and cellular differences [see, e.g. 39-44], including varied antigen distribution, processing and presentation.

Though we believe there is merit to this argument, debate about the value and validity of pre-clinical research is beyond the scope of this article. Here, for the sake of argument, we accept current research ethics norms (as enforced by research oversight bodies) according to which evidence of successful pre-clinical research in non-human animals should precede first-in-human clinical trials.

The prospective Phase 1 CRISPR gene editing cancer trial is supported by (only) one non-peer reviewed pre-clinical study in genetically modified NSG mice infused with NY-ESO-1 Redirected CRISPR edited T Cells. The problem with this pre-clinical study is threefold, (1) use of a single (small) animal model, (2) in only one laboratory, and (3) in only one species.

In our view, there should have been several pre-clinical studies with independent replication by research teams that are financially disinterested in the outcome [44-46]. As well, some of these studies should have been in different (including larger) species of non-human animals. For example, the investigators could have validated the pre-clinical study using canines, which provide an attractive translational model and share with humans many features, including tumor genetics, molecular markers, histology, biological behavior, tumor progression and response to conventional therapies [47, 48]. In addition, pre-clinical studies using canines may allow for long-term assessment of efficacy and toxicity [48]. Finally, some of these animal models should have had cancers more similar to those afflicting patients with melanoma, synovial sarcoma, or multiple myeloma [44].

Importantly, the investigators stated that they had not performed toxicology studies in the one pre-clinical study in mice to test for safety, including clinical observation, weight, mortality, clinical pathology, organ weight, gross pathology, and histopathology, as well as to test for efficacy and biodistribution of the investigational cell product. Given that the primary endpoints of the first-in-human Phase 1 CRISPR gene editing cancer trial include patient safety, the investigators should have performed these tests prior to the RAC submission in order to generate a robust assessment of safety, rather than inform the RAC that these tests would be performed as a later date prior to the IND submission to the FDA.

No robust causal inferences for the first-in-human Phase 1 CRISPR gene editing cancer trial can be made on the basis of (only) one pre-clinical study using a single (small) animal model, in only one laboratory, and in only one species.

4. CREDIBILITY

Leaving aside, for the moment, concerns about the robustness of the pre-clinical evidence, there is reason to question the trustworthiness of the available information on the basis of which “relevant experts” are expected to assess the likely predictive value of the pre-clinical evidence marshalled to justify the move to first-in-human clinical trials [14: p.122].

According to Kimmelman, matters of potential concern include: (1) optimism bias which can result in the skewing of pre-clinical research findings; (2) financial conflicts of interest as when investigators hold patents related to the proposed clinical trial (this may result in bias, information non-disclosure, or a premature move to the clinical setting); and (3) publication bias resulting in the non-publication of results from pre-clinical research.

4.1. Optimism Bias

Kimmelman describes optimism bias as a conscious or subconscious tendency on the part of investigators to present their data in a favorable light. This bias may be unintentional as a result of excessive enthusiasm; or intentional as a result of decision-making about the management of data outliers and missing data [14: pp.110-131].

The pre-clinical study involved three discrete groups of mice injected intravenously with: (1) NY-ESO-1 transduced T cells alone (investigators name these NY-ESO-1.TCR); (2) NY-ESO-1 TCR and PD1/TCR α/TCR β triple knockout T cells (investigators name these NY-ESO-1 TCR, CRISPR); and (3) T cells alone [2]. The two study endpoints were (1) tumor size and (2) percent survival, with no assessment of behavioral outcome.

Examination of the graphical data presented to the RAC reveals that approximately 33% of the animals in the NY-ESO-1 TCR treatment group survived past 80 days. However, the corresponding graphical data on the study endpoint tumor size at 80 days in this same NY-ESO-1 TCR treatment group reveals no data point. It appears that the investigators have selectively reported outcomes with respect to tumor size in the NY-ESO-1 TCR treatment group, which is suggestive of optimism bias.

4.2. Financial Conflicts of Interest

Biomedical research conducted in academic institutions is now commonly intertwined with pharmaceutical and biotechnology industries as part of an innovation ecosystem [49]. In this way, academic institutions and investigators have embraced a new kind of entrepreneurship in which financial conflicts of interest may arise [50]. In an effort to manage such conflicts of interest, academic institutions and professional organizations have developed policies governing academic-industrial collaborations. For example, the 2000 “Policy of The American Society of Gene Therapy Financial Conflict of Interest in Clinical Research” stipulates that,
Financial conflicts of interest, however, are not limited to conflicts resulting from relationships with research sponsors. Technology transfer endeavors, mainly through patenting and licensing to commercialize academic research, now play a major role in biomedical sciences [e.g. 49, 52-56]. This may give rise to additional conflicts of interest as per the 2013 FDA document “Guidance for Clinical Investigators, Industry, and FDA Staff: Financial Disclosure by Clinical Investigators” [57]. The FDA advocates extensive disclosure by investigators about compensation received, proprietary interests in the tested product (including a patent or licensing agreement), equity interests in any of the research sponsors, significant payments (including grants), and reimbursements such as retainers for ongoing consultation or honoraria.

Dr. Carl H. June of the University of Pennsylvania is the scientific advisor for the proposed Phase 1 CRISPR gene editing cancer trial. Importantly, the draft consent form presented to the RAC (which includes information about conflicts of interest) disclosed that Dr. June “invented the technology used to expand your cells for this study and he receives significant financial benefit related to this. This technology is licensed to a biotechnology company called Life Technologies, and has been sub-licensed to Novartis.”

During the RAC proceedings, there was confusion on the part of RAC committee members as to whether Life Technologies and Novartis were funding this Phase 1 clinical trial. Dr. June confirmed that they were not funding the trial and that the investigators needed to “clean that language up.” Dr. June explained that the Parker Institute for Cancer Immunotherapy was the funding sponsor. There was no unambiguous disclosure statement, however, addressing the relationship between Life Technologies, Novartis, and the Parker Institute for Cancer Immunotherapy. As such, details regarding potential financial conflicts of interest resulting from relationships with research sponsors were not fully addressed during the RAC proceedings.

As well, Dr. June did not provide information regarding registered patents (or filed patent applications) related to experimental agents to be utilized in the proposed Phase 1 clinical trial. Nor did he divulge to the RAC the exact nature of the “significant financial benefit” he receives as a result of his invention. An independent search reveals that Dr. June is the inventor of a significant number of patents registered with the United States Patent and Trademarks Office in the field of methods and compositions for treatment of cancer in humans, including the administration of genetically modified T cells [58, 59]. Moreover, many of these registered patents are assigned to the global healthcare company Novartis AG.

Further, there was no disclosure to the RAC about whether Dr. June has equity, stock options or comparable arrangements in companies sponsoring or funding the trial, whether he receives gifts, ongoing consultation fees or honoraria, or whether he will be paid with respect to research participant recruitment. As well, there was no mention of any mitigation strategies that might be in place.

In an effort to address patent-related conflicts of interest, Kimmelman has recommended use of restrictive policies requiring: (1) disclosure of registered patents on experimental agents held by investigators to IRBs and research participants; (2) curtailed responsibilities for investigators who hold registered patent interests in an experimental agent (up to and including being presumptively barred from certain activities in the clinical trial); (3) management (including disclosure) of institutional interests in registered patents on experimental agents; and (4) disclosure to IRBs of all patent filings related to the experimental agent. The stringency of such policies are to be adjusted according to the trial and the patent [60].

Following on these recommendations, it would have been prudent for Dr. June to voluntarily curtail his responsibilities as scientific advisor of this Phase 1 clinical trial. In the alternative, the University of Pennsylvania could have presumptively barred Dr. June from certain activities such as patient interactions and selection, study design, data analysis, and other activities. Instead, the University of Pennsylvania Conflict of Interest Standing Committee has yet to determine whether Dr. June can participate in his proposed capacity, or in the alternative, if a management plan should be issued and agreed to by Dr. June.

In addition to individual conflicts of interest, there may be institutional conflicts of interest. Institutional conflicts of interest may arise when a financial interest of an employee of an institution, or of the institution itself, could affect or appear to affect the conduct, review, or oversight of research in ways that are potentially harmful to the obligations, the mission, or the values of the institution [61]. The draft consent form presented to the RAC stated that the University of Pennsylvania had a significant financial interest in the technologies being evaluated in the proposed Phase 1 clinical trial. As such, if these technologies were proven safe and effective, the University of Pennsylvania would financially benefit. The RAC was not provided with detailed information about institutional interests, financial or otherwise, in registered patents related to the experimental agent, or any mitigation strategies that might be in place.

4.3. Publication Bias

Publications that demonstrate a strong relationship between small sample sizes and large treatment effect or that withhold negative results may be a frequent occurrence in pre-clinical efficacy studies [e.g. 14: pp.110-131; 62]. These forms of publication bias, which distort the efficacy of pre-clinical interventions and manifest a lack of transparency, invariably result in an inability to reproduce scientific results. This not only complicates interpretation of the medical literature, it also violates the ethical obligation to only involve persons in research that contributes to knowledge [62].

According to the investigators, the one pre-clinical study in a small number of immunodeficient NSG mice (used to justify the first-in-human gene editing cancer trial) showed a large treatment effect and proved to be safe and efficacious.
This study has not yet been published in a peer reviewed journal, however. Peer review would have allowed for an independent scientific assessment of the relevant data. It is widely recommended that the publication of pre-clinical studies follow the Animal Research Reporting In Vivo Experiments (ARRIVE) criteria [63: p.19].

4.4. Summary of Concerns Regarding Credibility

Optimism bias, potential financial conflicts, competing interests between investigators, institutions and pharmaceutical companies, as well as a publication bias, raise serious concerns regarding the credibility of the pre-clinical study that provided an evidentiary basis for the move to the first-in-human Phase 1 gene editing cancer trial.

5. DISCUSSION

As noted at the outset, there are those who wonder (ourselves included) whether the proposed first-in-human Phase 1 CRISPR gene editing cancer trials are premature [64]. In an effort to address this issue we critically examined the quality of the pre-clinical evidence used to justify the first-in-human Phase 1 CRISPR gene editing cancer trial in the United States using available evidence presented to the RAC. Our analysis relied heavily on the four-part framework developed by Kimmelman for assessing translational distance between pre-clinical and clinical research and on the structured process subsequently developed by Henderson and Kimmelman for evaluating pre-clinical evidence in terms of potential threats to internal validity, construct validity, and external validity. In our estimation the scarcity and poor quality of pre-clinical evidence and the novelty of NY-ESO-1 Redirected CRISPR Edited T Cells warrants further study prior to any first-in-human trial.

Our analysis of internal validity suggests problems with methodological elements of the pre-clinical study including the small sample size, and the lack of a-priori power calculations, randomized allocation of animals to treatment, blinding of outcome assessment, dose-response relationships, and selection of appropriate control groups.

Construct validity is threatened as the pre-clinical study did not validate the hypothesis to be tested in the first-in-human Phase 1 clinical trial. The investigators should have tested the efficacy of lentiviral transduction and gene editing in T cells from cancer patients (including perhaps prospective research participants) with melanoma, synovial sarcoma, or multiple myeloma and thereafter used the same T cells in the pre-clinical animal study to determine the two study endpoints of (1) tumor size and (2) percent survival. Consequently, not all of the features of the pre-clinical study were held constant in the move from pre-clinical research to the Phase 1 clinical trial. There is no principled justification for not proceeding with an expanded pre-clinical analysis involving more than two cell sources and using cells that were from the target populations, namely, patients with melanoma, synovial sarcoma, or multiple myeloma, when testing the efficacy of the lentiviral transduction and gene editing. The potential heterogeneity of the cell populations further negates the possibility of robust causal inferences about the safety and efficacy of any research intervention.

External validity analysis indicates that limitations are further compounded by the use of no animal models other than mice and no independent replication. The fallacy of drawing inferences from (only) one pre-clinical study in mice to justify the move to a first-in-human clinical trial cannot be over-emphasized. Addressing these limitations would have been of potential scientific value and could usefully have informed clinical trial design.

Kimmelman has stated that the most effective way to minimize translational distance (i.e. to ensure the narrowest possible inferential gap) between pre-clinical research and clinical research is to have all features of the relevant pre-clinical studies held constant in the move to first-in-human trials. Importantly, our analysis highlights significant problems with internal validity, construct validity, and external validity as these relate to features of the one pre-clinical study in mice. As regards the proposed first-in-human gene editing cancer trial in the United States, in an ideal context, the pre-clinical research should have proceeded in the following step wise fashion. There should have been research to transduce and gene edit T cells from healthy donors, followed by research using a similar or improved protocol to transduce and gene edit T cells from cancer patients (ideally patients with melanoma, synovial sarcoma, or multiple myeloma). Thereafter, transduced and gene edited T cells from cancer patients should have been cell sorted to obtain a homologous population of T cells expressing NY-ESO-1 TCR with endogenous TCR α, TCR β and PD-1 disruptions. Next, this homologous population of T cells from cancer patients should have been introduced into a mouse model that had either melanoma, synovial sarcoma, or multiple myeloma like tumors in an anatomical location that mimics the human condition in order to assess safety and efficacy. In turn, this should have been followed by similar research in other (including larger) animal models. As best we can tell, these steps were not followed prior to seeking RAC approval to initiate the first-in-human Phase 1 CRISPR gene editing cancer trial.

Finally, an appraisal of credibility brings into question the trustworthiness of the publicly available information. For example, small sample size and a missing data point in the analysis of tumor size may indicate an optimism bias. Potential conflicts of interest cannot be overlooked given the significant individual and institutional financial interests related to patents, licensing arrangements with industry and milestone payments. Finally, a publication bias arises due to the small sample size and the reported large treatment effect in the pre-clinical study, as well as the lack of peer review.

As such, all that can be concluded from the one pre-clinical study in mice is that the investigators’ methods were lacking in scientific rigor and did not meet the ethical requirement of scientific validity.

CONCLUSION

The myriad problems of scientific validity with the pre-clinical study presented to the RAC in support of the move to a first-in-human Phase 1 CRISPR gene editing clinical trial for cancer is concerning. This arguably calls into the question the confidence expressed by international groups and organizations in existing governance mechanisms for human somatic cell gene editing.
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For example, in December 2015, the organizing committee of the first international summit on human gene editing (sponsored by the United States National Academy of Science, the United States National Academy of Medicine, the Royal Society and the Chinese Academy of Science) released *On Human Gene Editing: International Summit Statement* at the close of the meeting [65] [F.B. was a co-author]. This statement unequivocally endorsed the view that regulators are capable, on the basis of past experience with research involving gene transfer, to weigh the risks and potential benefits of clinical trials involving somatic cell gene editing:

“[b]ecause proposed clinical uses are intended to affect only the individual who receives them, they can be appropriately and rigorously evaluated within existing and evolving regulatory frameworks for gene therapy, and regulators can weigh risks and potential benefits in approving clinical trials and therapies.” [Emphasis added] [65: conclusion no.2]

Similarly, fourteen months later, the February 2017 report *Human Genome Editing: Science, Ethics and Governance* [66] authored by the United States National Academy of Science and the United States National Academy of Medicine assuredly concluded:

“… that clinical trials of genome editing in somatic cells for the treatment or prevention of disease or disability should continue, subject to the ethical norms and regulatory frameworks that have been developed for existing somatic gene therapy research and clinical use to treat or prevent disease and disability.” [Emphasis added] [67: p.2]

This conclusion is formalized as recommendation 4-1:

“Existing regulatory infrastructure and processes for reviewing and evaluating somatic gene therapy to treat or prevent disease and disability should be used to evaluate somatic gene therapy that uses genome editing.” [66: p.61]

Our analysis, however, suggests that confidence in existing regulatory processes may be misplaced.

One possible explanation for the misplaced confidence in existing regulatory processes has to do with context. Those responsible for the interpretation and application of the regulatory framework in the United States, no doubt, are mindful of the fact that Chinese researchers have a head start. Their first-in-human gene editing trial for cancer is well underway, the first patient having been enrolled in October of 2016. In the Fall of 2016, Dr. June, the scientific adviser for the trial in the United States, said explicitly “I think this is going to trigger ‘Sputnik 2.0’, a biomedical duel on progress between China and the United States” [64]. This prediction, not surprisingly, made the headlines [68, 69]. Although Dr. June believes that this form of competition “usually improves the end product”, the pivotal ethical question is “at what cost?” If the move to first-in-human clinical trials is premature, as our analysis suggests, then trial participants will have been put at risk for no potential benefit.

Here we side with the American geneticist and Nobel Laureate Hermann Joseph Muller who believed that individual countries should not be encouraged to develop genetic or biomedical sputniks. In a 1957 address at the University of New Hampshire entitled, "Man’s Responsibility for His Genetic Heritage" [70], Muller hopefully asserted,

“Fortunately, men will in all probability have joined into one world community before these techniques come into widespread use. For if the people of one nation were to apply them intelligently and extensively even a few decades before the rest of the world did so, they would be able soon afterwards to rise to a so much higher level of capability as to make them virtually invincible. The world cannot afford to allow to individual countries their separate genetic sputniks!” [as reprinted in: 71: p.3]

As for the proposed first-in-human Phase 1 CRISPR gene editing cancer trial in the United States, we side with Mildred Cho – one of the RAC reviewers for this trial. Cho asserts in her review:

“... it seems to me that the novelty of triple editing to disrupt endogenous TCR and PD-1 in combination with adoptive T cell transfer warrants more animal studies in order to better anticipate the effects of PD-1 disruption and of the bulk transfer/‘pick the winner’ strategy." [72: p.12]

Subsequently, in a commentary on this proposed research, Cho is reported to have said that there is only so much investigators can learn from animal models and that at some point “we have to take a leap of faith” [cited in: 73]. The critical issue, however, is how far should investigators be willing to jump… The smaller the distance, the greater the likelihood of a safe landing. This explains the ethical obligation imposed on investigators to do everything reasonably possible to narrow the inferential gap between what is known from pre-clinical research and what is proposed for first-in-human clinical trials. Initiating first-in-human CRISPR gene editing cancer trials is an important step on the path to developing safe and effective preventive and therapeutic interventions for current and future patients. It is not a step to be taken lightly. The hoped-for knowledge is not to be obtained at any and all costs to current patients who consent to become research participants.

In our view, the move to first-in-human Phase 1 CRISPR gene editing cancer trials in the United States, on the basis of pre-clinical evidence presented to the RAC, is premature insofar as it makes the leap of faith a leap too far. Moreover, this leap cannot be justified by claims of urgent medical need.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.
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