EDITORIAL

Reverse translation: the key to increasing the clinical success of immunotherapy?

Therapeutic testing in animal models has been the cornerstone of translational medicine. However, this trend is starting to change in favour of non-animal alternatives. Considering the high failure rates of forward translation from animal models to human application, the above paradigm shift is definitely welcome. But the enthusiasm toward this progress should not become the basis for completely replacing animal testing because the reliability and representativeness of non-animal alternatives still needs more investigation. And this particularly applies to analyses of the immune system and validation of immunotherapies. In this editorial, we discuss the application of reverse translation as a possible key to robustly connecting human immune data with animal testing to increase the benefit-to-risk ratio of translating immunotherapies toward prospective clinical trials.

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Translational medicine is currently experiencing a significant paradigm shift in terms of its operational features. Classically, therapeutic or drug testing in animal models like rodents, pigs, sheep, monkeys has been the cornerstone of translational medicine by serving as the penultimate step before human testing [1]. However, this trend is starting to rapidly change because a number of regulatory authorities are trying to move away from animal testing due to ethical concerns as well as low success rates of clinical trials over the last few decades [1]. For example, the recent decision by US Food and Drug Administration (FDA) to replace the word "animal" with "nonclinical tests" has rapidly paved the way for prioritizing the use of non-animal (alternative) therapy/drug testing methods [1]. Such methods include, but are not limited to, cell-based testing, patient-derived xenograft (PDX) models, organoid or organotypic explant cultures, organs-on-a-chip and in silico modelling [1–5]. Herein in silico testing is primarily driven by either artificial intelligence (AI)-driven approaches like machine/deep learning or advanced pharmacodynamic computer modelling.

Considering the poor success rate of forward translation from animal models to human clinical application [6], the above paradigm shift is definitely welcome. However, the enthusiasm toward this progress should not become the basis for completely replacing animal testing in favour of non-animal alternatives because the reliability, reproducibility and representativeness of the non-animal alternatives still needs more investigation [1]. And this caveat is particularly applicable to analyses of the immune system and validation of immunotherapies. For instance, PDX models or organoids may not successfully retain the entire native immune-microenvironment of a diseased tissue [5, 7–10]. And while this limitation can be somewhat overcome by organotypic explants or organs-on-a-chip yet they may only sustain a relatively transient immune milieu whose propagation and preservation characteristics require more research [7, 10]. Finally, although in silico testing can help gain a lot of novel insights from multi-omics 'big data' [11] yet it is likely to remain only as efficient as the pre-existing training/validation datasets used to create these approaches. Thus, it is not yet clear whether in silico approaches can reveal something fundamentally novel about the mechanisms behind immunological processes that we ourselves do not yet understand based on experimental approaches.

Moreover most non-animal alternatives are intensively focussed on the diseased tissue characteristics and behaviour. But a number of multi-organ immune processes independent of the diseased tissue can be highly instrumental for the success of immunotherapy and such processes cannot be reliably or feasibly modelled by some of the current non-animal testing methods. Due to high amount of clinical data availability, this has been particularly evident for cancer immunotherapy. For instance, while organoids or organotypic explant cultures can account for tumour-associated T cell dynamics or tumour-associated 'cytotoxic revival' of T cells after immunotherapy [8] yet these approaches may not sufficiently capture: (I) anticancer T cell repertoire in extra-tumoural milieu like peripheral blood, normal-adjacent tissues and/or draining lymph nodes that may be instrumental in avoiding metastasis or tumour relapse [12, 13]; (II) accountability for intra-tumoural infiltration of novel T cell subpopulations from extra-tumoural milieu (that were previously absent in the tumour) i.e., 'clonal replacement' [12]; (III) finally, stem-like progenitor T cells may be more enriched in lymph nodes or peripheral tissues rather than tumours due to 'T cell exclusion' [12, 14], and thus may not be captured with tumour-specific organoids/explants [10, 15]. These extra-tumoural dynamics and their cross-talk with tumour-associated T cell repertoire are highly predictive of immunotherapy responses in cancer patients [12] and thus need to be accounted for during immunotherapy testing. Thus, it is evident that only animal models may allow the analyses of such multi-organ and extra-diseased tissue processes in a dynamic manner.

Nevertheless, one cannot simply ignore the dismal success rate of current translational medicine based on animal models. Afterall, work-horse animal models of immunology like rodents are differentiated by an evolutionary gap of at least 65 million years from humans which has created significant variations and evolutionary divergences for both innate and adaptive immunity that creates severe bottlenecks for translation of immunotherapies [16]. This is particularly applicable to immune cell

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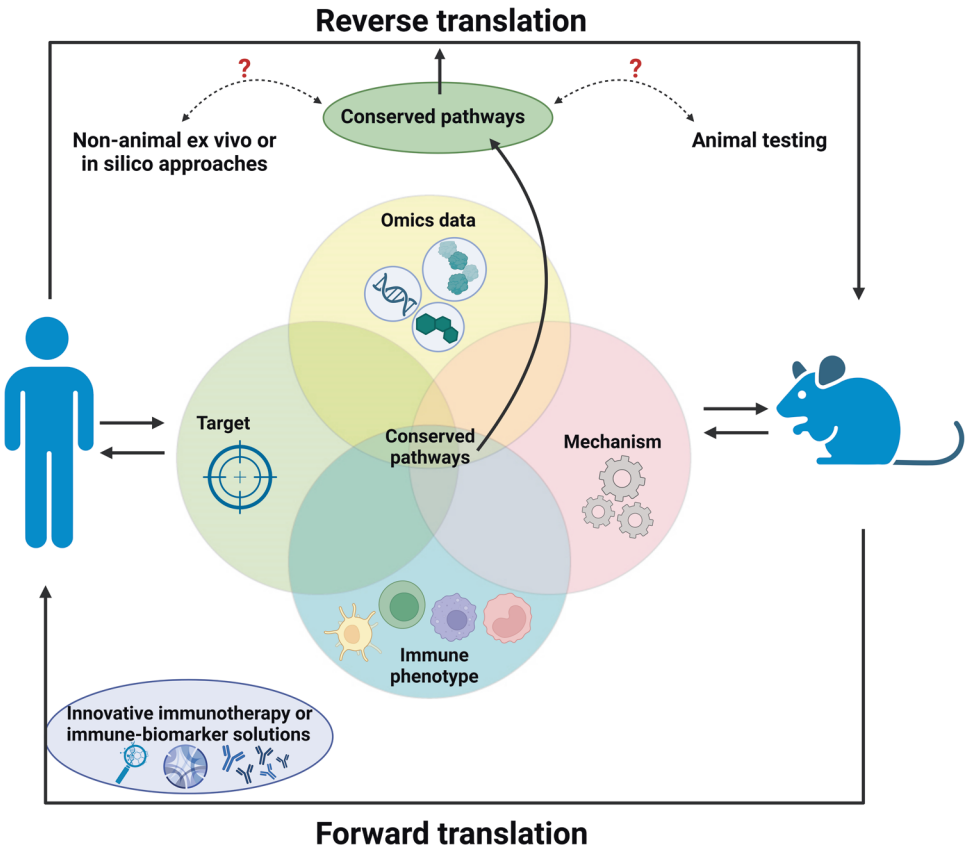

Fig. 1 A schematic overview of mixed reverse and forward translational approaches for immunotherapy. The bottom part depicts the typical forward translational approach often pursued by most researchers. The top part depicts a much needed reverse translational approach to increase clinical success rates. The middle part captures the intersection of different research directions and orientations that can be pursued to simultaneously enable both reverse and forward translational approaches.

subset heterogeneity, cytokine/chemokine biology, anti-microbial defence systems including Toll-like receptors, NK cell biology, myeloid phagocytic and co-simulation systems, B/T cell repertoire dynamics, type-1 vs. type-2 orientations of the T cells, γδ T cell biology, and vascular-immune cross-talk [16].

So what is the possible solution that balances the usage of animal testing with the need to increase the success rate of clinical translation for immunotherapy? One robust solution might be reverse translational approaches using in silico and ex vivo testing to bridge the human phenotypes with animal testing before channelling the final results back into the conventional forward translation [17, 18] (Fig. 1). This mix of reverse and forward translation of fundamental and translational immunology research has a high chance of increasing the clinical success rates of immunotherapy [17]. But, to be successful, such approaches need to achieve three critical steps (Fig. 1): (I) generation of appropriate human patient 'big data' cross-connected with clinical response variables, possibly aided by non-animal testing approaches to create an integrated picture of the human 'immunome' for a particular disease; (II) multi-dimensional computational and immunology approaches to bridge the evolutionary gap between humans and mouse; (III) tailoring of these approaches to appropriate mouse models that are as close as possible to the context of human disease-immune cross-talk.

Such reverse translational approaches would essentially work backward to uncover the preclinical (evolutionarily-conserved) mechanistic basis for clinically important (broad) immune phenotypes [17] (Fig. 1). In time such approaches may assume a cyclical nature, in which each new human observation might stimulate new testable concepts or hypotheses that help create the next innovative immunotherapy or immune-biomarker solutions for forward translation toward a novel human clinical trial [17] (Fig. 1). Such reverse translation can profoundly improve: immunotherapy validation or re-purposing efforts, patient-tailored immunotherapy, understanding of the reasons behind success or failure of specific immunotherapies, and uncovering the impact of human-specific immuno-variability on immunotherapy responses [17]. These efforts may also uncover the exact threshold beyond which animal testing is not useful hence uncovering the immune niches that non-animal testing must address. Together such combination of reverse translational approaches and non-animal testing can help improve the benefit-to-risk profile of different immunotherapies and provide some preliminary immune-biomarkers for pre-selection of patient sub-populations most likely to respond to such immunotherapies [17]. Herein, in silico testing needs further optimization to allow better modelling of human physiology-relevant pharmacokinetics, therapeutic response variability and pre-anticipation of potential adverse events in the setting of novel immunotherapies. This may help increase the success rate of both first-in-human (dose escalation) clinical studies and early phase clinical trials for estimating pharmacodynamic and preliminary clinical response variables.

Finally, in coming future, the biggest gap-in-knowledge that still needs to be properly overcome is understanding the origins of the population immunogenetics-driven variability in immunotherapy responses inherent to different human populations and the confounding influence of age, gender, ethnicity and other such human-specific criteria. Current animal or non-animal testing methods are not completely adapted yet to properly account for these variabilities on a large scale. Here, the integration of the electronic health records of various

patients with in silico/ex vivo testing could be the key to connect real-world human variability with forward translational immunotherapy efforts.

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ADDITIONAL INFORMATION

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