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Recent advancements and applications of humanised mouse models in preclinical immuno-oncology

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Introduction

Humanised mice, or human immune system (HIS) mice, are terms used to describe immunodeficient mice reconstituted with a human immune system. They are increasingly used for modelling the human immune compartment and are a powerful translational tool for investigating the human immune response in disease states. The ability to investigate human immune behaviour in rodent models offers a new generation of *in vivo* investigations which until now have been futile in murine models without human immune representation. Here we review the humanised mouse platforms and technology available, and their potential applications in immuno-oncology safety, efficacy, and new modalities therapy development.

Humanised mouse model platforms

Three major humanised mouse models have been used to examine the human immune response in immunocompromised mice: the hu-PBL (human Peripheral Blood Lymphocytes) model, the hu-SRC (human Stem Repopulating Cell) model, and the BLT (Bone marrow, Liver, Thymus) model. Each model has its own advantages and disadvantages, described in detail below (Figure 1) and a summary of the models can be found in Table 1.

Hu-PBL model

The hu-PBL model has been extensively used for the study of mature immune responses and is the simplest and most cost-efficient method of humanisation. Human leukocytes can be easily isolated from peripheral blood, spleen or lymph nodes in larger numbers than stem cell and fetal samples required for the other two platforms. Isolated leukocytes can then be engrafted by intravenous (IV) or intraperitoneal (IP) injection into adult immunodeficient mice, which may be pre-conditioned with a sub-lethal dose of irradiation (Figure 1). This model avoids significant delay in engraftment as the human leukocytes injected are already mature. Human leukocytes can be found circulating in the murine peripheral blood within days after injection and maintained for up to 4-6 weeks. Human T cells are the main population present in this model, with an activated memory/effector phenotype [1, 2]. Additional subpopulations including human B cells and myeloid cells are present, but at much lower levels. This is likely due to the lack of human specific cytokines required for their survival and the dominant expansion of T cells [3, 4]. The main disadvantage of this model is the rapid development of graft-*versus*-host disease (GvHD), directly correlated with the levels of human

activated T cells present in the host due to their recognition of the murine environment. Hence, the experimental window of this model is limited to short-term studies [5]. However, utilisation of new strains of mice deficient in the major histocompatibility complex (MHC) class I and/or II, can delay GvHD and increase survival to widen this experimental window [6].

Hu-SRC model

The hu-SRC model is established following injection of human haematopoietic stem cells (HSC) in immunocompromised mice engrafted either as adults [7] or newborns [8]. For this model, pre-conditioning with sub-lethal irradiation is essential. HSC cells can be obtained from cord blood [9], bone marrow (BM) [10, 11], fetal liver [11] or BM-derived HSCs mobilised into the peripheral blood with growth stem cell factor (GCSF) [12]. Cells are injected IV or intrafemoral (IF) in adult mice and either IV (facial vein), intracardiac (IC) or intrahepatic (IH) in newborns [2] (Figure 1). In this model, a diverse repertoire of cell populations are generated. Engrafting newborn or young mice (up to 4 weeks) allows accelerated generation of T cells in comparison to adult mice [13]. The main caveats of this model are the time taken to establish this model (typically 16 weeks or more), a lack of a functional B cell compartment which is in part due to inadequate CD4 helper functions, and lastly impaired antigen response by the HIS [14-16]. This is partly due to the absence of human primary lymphoid organs and therefore limited differentiation of human cells in the mouse environment, and secondly due to the lack of human Human Leukocyte Antigen (HLA) on thymic epithelium and therefore a lack of HIS, specifically T cells, education. Advances in the development of new strains which incorporate human growth factors and cytokines through genetic manipulation, summarised below (Table 1), may help to overcome some of these deficiencies.

BLT model

In the BLT model, mice are surgically transplanted with fragments of human fetal liver and thymus under the kidney capsule of sub-lethally irradiated immunocompromised mice. This is followed by an IV injection of autologous HSC cells [17] (Figure 1). This model allows systemic repopulation of multiple lineages of human immune cells including T, B cells, monocytes, macrophages, and dendritic cells. Superior to the other models is the formation of secondary lymphoid organs which aid in HIS education and therefore enhance immune responses to antigens [18, 19]. Because human T cells are educated in autologous thymic tissues in the BLT model, it has been important in the investigation of human T cell

development [20, 21]. Furthermore, it has had a great impact on the study of infectious diseases, especially in human immunodeficiency virus (HIV) infection, as this model improves the colonization of lymphoid organs, together with the human reconstitution of the mucosal and gastrointestinal tracts [19]. In this model, BLT mice can recapitulate mucosal transmission of HIV via vaginal and rectal routes [22]. Nevertheless, these mice exhibit a higher incidence of GvHD compared to the hu-SRC model, some of them earlier than 20 weeks post-transplantation [23].

Immunodeficient strains

A variety of immunodeficient mouse strains can be used above part of the models described above to host human immune cells, each appropriate for different experimental aims. A number of immunodeficient strains have been developed through genetic manipulation of *Prkdc*^{scid} and the recombination activating gene (RAG) to ablate adaptive lymphocytes [24]. Immunodeficient *scid* and RAG mice are crossed with non-obese diabetic (NOD) mice which have a dysfunctional innate immune compartment. The introduction of an IL-2Rγ knock-out blocks the signalling pathways of a diverse repertoire of cytokines and therefore results in the absence of functional NK cells. The main immunodeficient platforms include NSG [8, 12], NOG [7, 25], NRG [26, 27], and BRG [28, 29]. Their genetic background, full names, acronyms, and descriptions of their acronyms are summarised in Table 1. Comparison of human CD45 engraftment in NSG, NOG and BRG show that NOG and NSG have higher engraftment levels than BRG [30]. In this study, higher engraftment of cord blood reconstituted mice was reported in NSG mice compared to three other immunodeficient strains. Furthermore, higher engraftment in NSG females has also been described [31].

A wide variety of NSG strains have been developed in recent years to overcome issues such as GvHD, lack of HLA-specific responses, and lack of innate immune cell survival. Several of these NSG strains have been previously reviewed by Shultz, L.D. et al (2012) [29]. In this review, commonly used strains [5, 6, 32-42] are summarised in Table 1 and some more recent advancements in immunodeficient platforms are described below [43-48].

The survival of natural killer (NK) cells and the myeloid compartment has been a major limitation in humanised mouse models. Recently, new immunodeficient strains have been developed to address this shortfall. IL-15 is imperative for NK cell development and this has been exploited in order to improve NK cell engraftment in immunodeficient models [43, 44].

NSG-IL15, with transgenic IL-15 expression, show enhanced $CD56^+$ NK cell development in blood, spleen, and bone marrow [43]. SRG-15 is another immunodeficient mouse model which supports NK cell development [44]. A human SIRPα knock-in on a BRG background (SRG) was crossed with a human IL15 knock-in mouse to generate SRG-15 mice. Mice humanised with $CD34^+$ fetal liver haematopoietic stem and progenitor cells (HSPC), supported $CD8^+$ T cell, NK cell, and innate lymphoid cell (ILC) development. In this model, higher circulating NK cells were found in the blood compared to SRG and NSG without IL-15 expression. Furthermore, both major NK cell subsets, $CD56^{bright}$ $CD16^-$ and $CD56^{dim}$ $CD16^+$ NK cells, were present in the blood and spleen of SRG-15 mice 7 week post-engraftment. These NK cells shared phenotypic similarities with circulating human donor NK cells and were highly functional as shown by evident tumour infiltration. Another model, MISTRG mice (Table1) also support myeloid and NK cells [45]. However, MISTRG mice develop anaemia early and therefore their lifespan is a limiting factor in experimental design.

In order to increase engraftment, immunodeficient mice are commonly irradiated before reconstitution with the human compartment. However, this increases hematopoietic, gastrointestinal, and neurological side effects associated with radiation. NBSGW mice (Table 1) support $CD34^+$ HSC engraftment without irradiation. These mice are generated through crossing NSG mice with the C57Black mice homozygous for c-Kit, the stem cell factor (SCF) receptor required for haematopoiesis [46].

Although advances have been made to increase HLA restricted responses in humanised mouse models, they have been restricted to introducing specific HLA molecules. A new immunodeficient model, HUMAMICE (Table 1), express all human HLA molecules instead of mouse MHC molecules [48]. HUMAMICE were generated through depletion of murine Rag 2 allele, IL2rγ allele, and the perforin allele (Perf), from a Sure-L1 background; mice expressing human HLA molecules. HUMAMICE reconstituted with HLA-matched ($HLA\text{-}A2^+DR1^+$) PBMCs showed T and B cell survival up to 12 weeks and showed evidence of functional humoral immunity demonstrated by IgG and IgM responses to immunization. Furthermore, there was no evidence GvHD compared to NSG class II knock out (KO).

Pathology of humanised mouse models

Limited information is available concerning the morphological aspects of tissue repopulation and the prevalence of histopathological lesions in immunodeficient mice engrafted with a human immune compartment. Functional defects of the immune system in immunodeficient mice are paralleled by abnormal development of the primary and secondary lymphoid organs, which vary structurally across strains [49]. For example, NSG mice, one of the most common options for successful humanisation [7, 50, 51], exhibit small spleen, thymus, and lymph nodes. Furthermore, these organs lack lymphoid structures and are composed almost exclusively of reticular stromal cells [12, 29, 49, 52]. Following transplantation of human cells, factors such as donor cell source, features of the recipient host system, and engraftment methodologies have a great influence on the extent, cell composition, and morphological aspects of reconstitution in the different organs [13]. The majority of murine models of human immune engraftment exhibit variable proportions of multilineage repopulation in the hemolymphatic organs. Here, human cells form variably sized aggregates that in general do not fully recreate the lymphoid tissue architecture typical of immunocompetent organisms, nor form distinctive lymphoid structures such as lymphoid follicles, germinal centres in the spleen or lymph nodes, nor lead to identifiable cortical and medullary structures in the thymus [17, 26, 53]. Repopulation of the latter appears to be more efficient in immunodeficient mice transplanted shortly after birth, whilst thymy of mice engrafted in adult age are alymphoid exhibiting hypoplastic cysts and are less prone to support human cell engraftment [13, 54].

Mice engrafted with human cells usually develop xenogenic GvHD within a few weeks post-transplantation [55, 56]. Prevalence, onset, and the morphological features of GvHD vary greatly across the various experimental models. However, GvHD occurs with high incidence and severity in those models where mature T cells represent the primary population of engrafting cells, in line with the pathogenesis of this immune-mediated condition in which activated T cells play a crucial role [57-59]. Clinically, GvHD occurs as a generalised wasting syndrome leading to progressive body weight loss and/or a severe skin condition characterized by alopecia; the latter is associated histologically with dermatitis and scleroderma [60, 61]. GvHD can however manifest also as a systemic inflammatory reaction affecting multiple organs including the hemolymphoid organs, liver, lungs, and several others

[60, 62]. Mice exhibiting symptoms of GvHD are often excluded from studies, although little is known concerning the actual prevalence of subclinical GvHD within each single model. The opportunity to examine T cell composition and reconstitution of lymphoid organs using flow cytometry and histology together is important to identify individual mice affected by GvHD, which could represent a confounding effect when interpreting engraftment and experimental data [29].

New technologies for advanced phenotyping and analysis of humanised mouse models

Flow cytometry, which uses fluorescent markers to identify cells, is a convenient, cost-effective method for evaluating human cell engraftment in humanised mice. Peripheral blood, spleen, bone marrow and other tissues can easily be processed to analyse percentages of murine and human $CD45^+$ cells. This can be extended to evaluate other human immune subsets as T cells (CD3, CD4, CD8), B cells (CD19), macrophages (CD68), and neutrophils (CD15) [63, 64]. Flow cytometry technology has advanced in recent years, with capabilities to measure an increasing number of parameters. New flow cytometers such as the BD FACSymphony™ system allows measurement of up to 50 different parameters on a single cell. To complete and validate the characterisation of human immune reconstitution by flow cytometry, immunohistochemistry (IHC) offers spatial context and histopathology to better understand tissue homing of human cells and identify instances of GvHD which must be considered in data interpretation [65-67].

Despite technological advances, the evaluation of human immune cells by flow cytometry and IHC is limited by technical constraints. Most flow cytometers are limited to 12-18 parameters per single sample, with the exception of BD FACSymphony™, and issues with spectral overlap make analysis difficult. Furthermore, the possibility of multiplexing on IHC is even lower (Table 2). To properly describe and understand the complexity of these mouse models, new high dimensional parameter analysis tools are essential.

A new phenotyping platform has been developed that couples flow cytometry with mass spectrometry known as mass cytometry. Mass cytometry offers single-cell analysis of up to 50 simultaneous parameters without spectral overlap [68, 69]. Cell surface and intracellular markers are coupled with stable metal isotopes, which are identified by mass through time-

of-flight (TOF) and linked to each single cell by a cell-ID incorporated before acquisition. Many of the antibody-metal isotope pairs are available and customisable from Fluidigm.

Mass cytometry has become a popular tool for deep immune profiling across innate [70-72] and adaptive immune subsets in humans [73-75]. More recently, Herndler-Brandstetter, D., et al (2017) performed deep immunophenotyping using 33 parameter mass cytometry of NK cells engrafted in SRG-15 humanised mice [44] .This supports the power and future scope of using mass cytometry to phenotype humanised mice.

Imaging Mass Cytometry™ (IMC™) performed on the Hyperion™ Imaging System using metal-tagged antibodies empowers simultaneous imaging of up to 37 protein markers at a time [76]. IMC combines a precisely directed laser beam focused on 1µm tissue section to collect biological samples stained with metal-tagged antibodies and directs these tags for analysis by cyTOF technology described above, while preserving the information in tissue architecture and cell morphology. This can be done on frozen and formalin fixed paraffin embedded (FFPE) tissue sections [77, 78].

In addition to mass cytometry, single-cell transcriptomics has led to new insights into the immune system. A combination of phenotypical (mass cytometry) and transcriptional (single cell RNA-sequencing) profiling have been used by Winkels et al (2018) to define an atlas of the immune repertoire in a murine atherosclerosis model, revealing new immunologic mechanisms and cell-type-specific pathways [79].

The high throughput capabilities of multi-parameter flow cytometry, mass cytometry, single cell transcriptomics, and IMC allows powerful high content analysis. T-distributed stochastic neighboring embedding (t-SNE), spade, and citrus algorithms offer visualisation of multi-dimensional data which can quickly identify subtle changes in immune populations. Several platforms can be used for this analysis, including Cytobank [80], FlowJo, R-studio, and MATLAB. Furthermore, Fluidigm have coupled their human immune monitoring kits with an automated gating software which uses probability state modelling and Cen-se algorithms to effectively and rapidly analyse 25 key immune subsets. In addition to the informative images acquired by IMC, quantitative analysis can be performed using various image analysis software such as Halo, cell profiler, and HistoCAT. The development of a multimodal approach with mass cytometry, IMC, and single-cell RNA-sequencing coupled with high dimensional analysis platforms supports next generation analysis of humanised mouse models with great

implications for their use in preclinical studies. Some of the promising applications of humanised mouse models in preclinical immuno-oncology studies are discussed below.

Preclinical applications of humanised mouse models

Using humanised mouse models for preclinical toxicology

The detrimental impact of cytotoxic and immunomodulatory drugs on the human immune system has long been a cause for concern with regards to the safety profile of therapeutic agents. Traditionally, toxicity testing of new small molecule drug candidates is undertaken in healthy preclinical species in clean housing facilities. Thus, although the drug candidate is pharmacologically active in the chosen toxicology species, translation of the pathway of interest and the immune context of the human patient population can result in adverse events manifesting in the clinic that were not evident preclinically. For biologics, preclinical testing is further limited by the high specificity of the drug product, so that the effect on the immune system of lower mammalian species may not recapitulate the likely outcome in humans. A potential solution to this challenge has been for the tests to be routinely conducted in non-human primates. However, this approach does not fully address inter-species differences and is additionally associated with significant ethical and economic considerations.

Reconstitution of immunocompromised mice with a human immune system has the potential to provide a step change in the prediction and translation of adverse events. Humanised mouse models have been used successfully to assess bone marrow toxicity and haematotoxicity of cytotoxic oncology treatments [81]. More recently, the opportunities afforded to novel drug development by humanised mouse models has been explored further, particularly with regards to human-specific drug modalities where preclinical models are limited. Humanised mice have been utilised to investigate the efficacy of human checkpoint inhibition [82] and the common adverse events observed with chimeric antigen receptor (CAR) T therapies. Humanised mouse models have provided mechanistic understanding for the cytokine release and neurotoxicity which occur with CAR T therapies, thus providing opportunities to mitigate these adverse events [83]. Indeed, there are exciting opportunities for humanised mouse models to provide a translatable, preclinical safety assessment of cell therapy approaches, where standard toxicology testing is unsuitable.

An area of drug safety that is particularly challenging to model preclinically are the immune related adverse events (irAEs) observed with immune-oncology agents, such as checkpoint inhibitors [84]. These adverse events do not manifest reproducibly in standard toxicological testing. In particular, combinations of checkpoint inhibitors can result in exacerbations of toxicity in the clinic, which would greatly benefit from more accurate preclinical modelling [84]. Humanised mouse models may provide a bridge to translate preclinical testing to the clinic, particularly given the opportunities to humanise mice with cells and/or human tissue fragments from patients to replicate the diseased state. For example, reconstitution of immunocompromised mice with cells from a human donor deficient in FOXP3 function resulted in manifestation of autoimmunity pathologies mimicking those noted in humans [85]. This promising finding raises the prospect that the humanised mouse model might, with optimisation and further development, be able to predict adverse immune events observed in the clinic. In particular, humanised mice may be predictive for preclinical tumour studies and adoptive cell therapy models which are discussed below.

Using humanised mouse models for tumour studies

Traditional mouse models do not recapitulate the heterogeneity and complexity of the original tumour and often lack predictive power, with over 80% of oncology drugs failing clinical trials [86, 87]. Patient Derived Xenograft (PDX) models have been shown to better retain tumour heterogeneity and have been used to study disease stabilisation, progression and response to chemotherapy treatments in a range of tumour types [88-93]. However, one major limitation of this model is the use of immunocompromised mice, hindering evaluation of tumour immune interactions and immunotherapies. Immunotherapies aim to treat cancer indirectly through reinforcing the anti-tumour immune response and can be administered in the form of vaccines, adoptive cell therapy (ACT), and immune checkpoint inhibitors. Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4), Programmed Cell Death Protein 1 (PD-1) and Programmed Death Ligand 1 (PD-L1) are common targets for immunotherapies and have shown promising yet highly variable response rates [94]. For example, response rates to PD-1/PD-L1 targeted immunotherapies, such as pembrolizumab and atelizumab, are seen in only 20-30% of patients despite demonstrated efficacy in variety preclinical tumour models [95]. Furthermore, immunotherapy-linked toxicity presents a significant challenge, with many patients required to manage emerging inflammatory disorders in addition to cancer. For

instance, Robert et al [96] found that metastatic melanoma patients treated with Ipilimumab (anti-CTLA-4) showed an 8.6% increase in survival over three years, however 56.3% of these patients developed grade 3 to 4 adverse effects such as colitis and hypophysitis. Findings such as these highlight the need for well-designed pre-clinical models that can accurately assess safety and efficacy of immunotherapies at varying doses, schedules, and combinations.

Humanised mouse models have been developed to more accurately depict the tumour microenvironment evolution and progression. As can be seen in Figure 3, Humanised PDX (HuPDX) models are typically established through humanisation of mice, as described earlier, followed by implantation of early passage xenograft tissue. HuPDX models have been applied to assess clinically approved immunotherapies in a variety of tumour types [97]. A key confounding factor is that in these studies the tumour and immune cells are obtained from different donors, thus overlaying an allogeneic immune response which can complicate interpretations of outcomes. Nonetheless, these models can be very informative. For example, Rosato et al [98] evaluated nivolumab (anti-PD-1) in a humanised model of triple negative breast cancer (TNBC) finding a positive correlation between PD-L1 expression and treatment response, similar to that seen in patients [99]. Humanised models also show great potential in assessing treatment toxicity. HuPDX models of hepatocellular carcinoma (HCC), which have been used to assess organ damage linked to ipilimumab, have been used to verify toxicity in the liver, lung, and kidneys [100]. Humanised models have also proved valuable in modelling drug refractory tumours and identifying effector mechanisms involved in treatment resistance [101]. However, the short lifespan of humanised mice makes these models ill-suited for longitudinal experiments as they do not truly reflect clinical scenarios such as drug discontinuation and reduction. Furthermore, it is often not feasible to reconstitute mice with autologous patient derived lymphocytes, especially in large-scale pre-clinical studies and many models rely on partial HLA-matched HSCs for reconstitution. Despite these limitations, humanised mouse models represent a novel and sophisticated tool in immuno-oncology research that will continue to evolve over time.

Using humanised mouse models for adoptive cell therapy

In the last ten years, T-cell therapies in which patient's immune cells are reprogrammed to express a CAR recognising tumour cells have shown great efficacy in the treatment of haematological cancers with remissions of up to 90% of cases [102]. Two T-cell therapies

based on a CAR recognizing CD19 (CAR19) have been approved by FDA (Kymriah™ and Yescarta™). They both direct autologous T cells to CD19-expressing tumour cells in acute lymphoblastic leukemia and B cell lymphomas (B-ALL) [122, 123].

Anti-tumour activity of CAR T-cells have been investigated *in vitro* and in *in vivo* models. To increase the clinical relevance of these studies, different mouse models have been employed. The xenograft mouse models have contributed to the development of better next-generation CARs with different signalling moieties [103-107] and "on-target/off-tumour" toxicity [108, 109], whereas the syngeneic mouse models have been instrumental in understanding the interplay between the tumours and the CAR T-cells in the context of an intact host immune system [110-112] and long-term engraftment of CAR T-cells [113]. The major disadvantage of these systems, however, is the lack of translatability to clinical outcomes, which is due to inability of these systems to mimic faithfully the patient's immune system or to the absence of other immune cells which contribute to the efficacy, persistence, and safety of CAR T-cell therapies.

Several pre-clinical and clinical studies are now ongoing to translate CAR T-cell efficacy to solid tumours; however, this is proving less successful due to several factors, including inefficient trafficking of CAR T cells into solid tumours and the presence of immuno-suppressive tumour microenvironments [114].

Humanised mice engrafted with tumour and CAR T-cells from the same donor should provide a more clinically relevant model for cancers. CAR-T cell therapy has been successfully modelled in humanised mice, recapitulating the post treatment immune changes observed clinically [115-117]. Recently, a humanised mouse model with a functional human immune system and an autologous tumour has been used to study CAR19 T-cell therapy for B-ALL pre-clinically [118]. This strategy avoided allogeneic responses by using fetal $CD34^{+}$ cells pooled from several donors to generate all the required human cells, and it recapitulated most of the clinical signs of CAR T-cell therapies. The question remains whether next-generation models will recapitulate mechanisms for tumour resistance and development of immune memory. Moreover, toxicity associated with cytokine release syndrome (CRS) observed in clinical trials of immunotherapies including CAR T-cells was not observed in these mice which may be due to undetectable levels of human IL-6, a primary driving force for CRS [119]. As this is a key

parameter in assessing the safety of CAR T-cell therapies, it is vital to predict CRS in a pre-clinical manner.

Together with the success for the CAR19 T-cell therapies, CRISPR technology has the potential to increase the efficacy and safety of reprogrammed T cells. Several publications have shown that knock-out of immune checkpoints (e.g. PD1 [120]) or insertion of CAR gene into the *TRAC* locus, so that its expression is controlled by the endogenous promoter [121], enhanced edited T-cell potency and clearance of tumour xenografts. To our knowledge, pre-clinical studies have only been conducted in immunodeficient mouse models in which the full potential and safety of CRISPR-edited T cells cannot be fully evaluated. This warrants further investigations into the use of humanised mice as a pre-clinical model for T-cell therapies.

Conclusions

Humanised mouse platforms are imperative for future investigations of the human immune response and have an essential part to play in investigating safety and efficacy of new immunotherapies. Together with the advancements in new strains and novel technologies to fully characterise these platforms, the expanding scope and application of humanised mice is emerging. Humanised mice have the potential to be an informative preclinical model. Toxicity issues observed in clinical studies, largely immune-mediated, that are not evident in preclinical animal model screening may be identified in humanised mice, reducing the adverse effects seen in patients. Furthermore, humanised PDX models offer a more representative human tumour microenvironment that better mimic the immune context in the patient. Adoptive cell and gene edited T cell therapy is becoming increasingly sought after in an effort to target tumours based on the donor's individual response. However, much is still unknown about the safety and efficacy of these personalised therapies and humanised mice offer a superior environment to investigate such issues. Together with advanced technologies emerging to phenotype and analyse humanised mice, humanised mice represent an exciting opportunity in the field of immuno-oncology and we can expect to see a surge in our knowledge around these models in the coming years.

List of Abbreviations

ACT	Adoptive Cell Therapy
Bcl	B cell lymphomas
BLT	Bone marrow, Liver, Thymus
BM	Bone Marrow
BRG	BALB/c.Rag.Gamma
CAR	Chimeric Antigen Receptor
CAR19	CAR recognizing CD19
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRS	Cytokine Release Syndrome
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
FFPE	Formalin Fixed Paraffin Embedded
GCSF	Growth Stem Cell Factor
GvHD	Graft versus Host Disease
HCC	HepatoCellular Carcinoma
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSC	Haematopoietic Stem Cells
HSPC	Haematopoietic Stem Progenitor Cells
HUMAMICE	HLA-A2$^{+/+}$/DR1$^{+/+}$/H-2-β_2m$^{-/-}$/IA$\beta^{-/-}$/Rag2$^{-/-}$/IL2r$\gamma^{-/-}$/Perf$^{-/-}$
hu-PBL	human Peripheral Blood Lymphocytes
HuPDX	Humanised PDX
hu-SRC	human Stem Repopulating Cells
IC	Intracardiac
IF	Intrafemoral
IH	Intrahepatic
IHC	Immunohistochemistry
IL2rγ	Interleukin 2 receptor gamma chain
IMC	Imagine Mass Cytometry

IP	Intraperitoneal
irAEs	Immune Related Adverse Events
IV	Intravenous
KO	Knock Out
MHC	Major Histocompatibility Complex
MISTRG	M-CSFh/h IL-3/GM-CSFh/h SIRPAh/m TPOh/h $Rag2^{-/-}$ $Il2rg^{-/-}$
NK	Natural Killer
NOD	Non-Obese Diabetic
NOG	NOD.Gamma
NRG	NOD.Rag.Gamma
NSG	NOD.Scid.Gamma
PBMC	Peripheral Blood Mononuclear Cells
PD-1	Programmed Cell Death Protein 1
PDL-1	Programmed Death Ligand 1
PDX	Patient Derived Xenograft
SCF	Stem Cell Factor
SRG-15	S.Rag2.Gamma-15
TNBC	Triple Negative Breast Cancer
TOF	Time of Flight
TRAC	T cell Receptor Alpha Constant
t-SNE	T-distributed stochastic neighboring embedding

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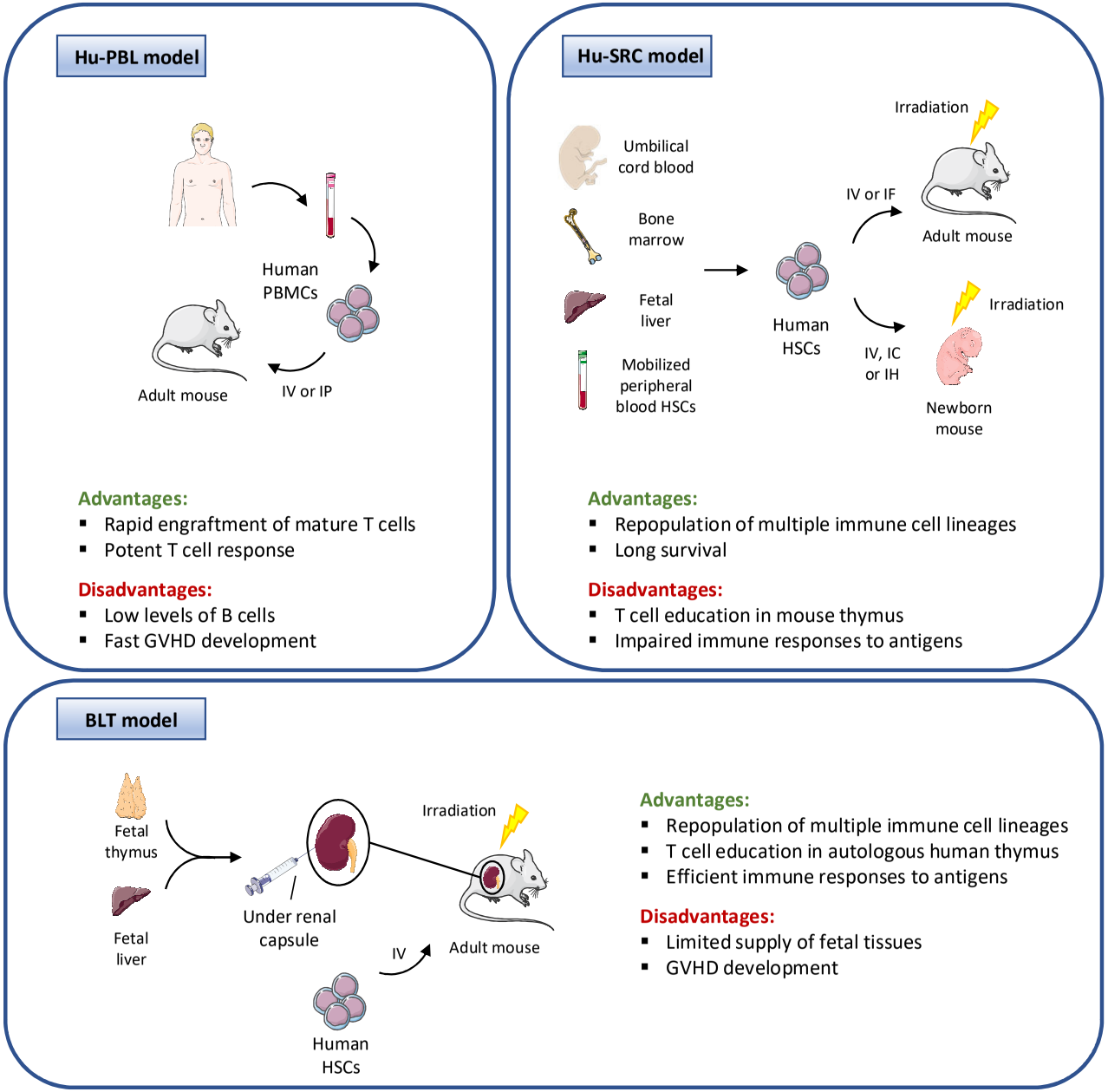
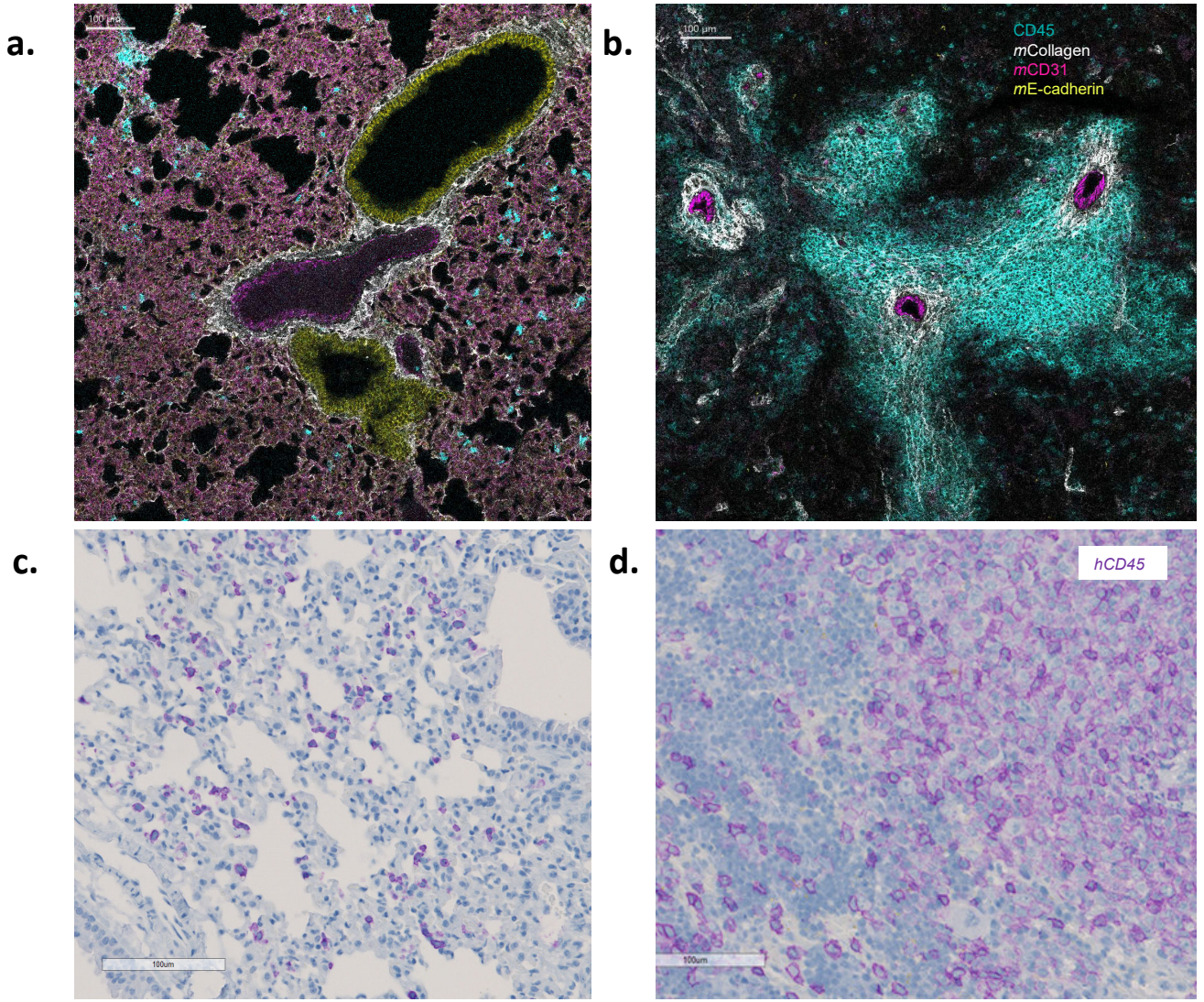
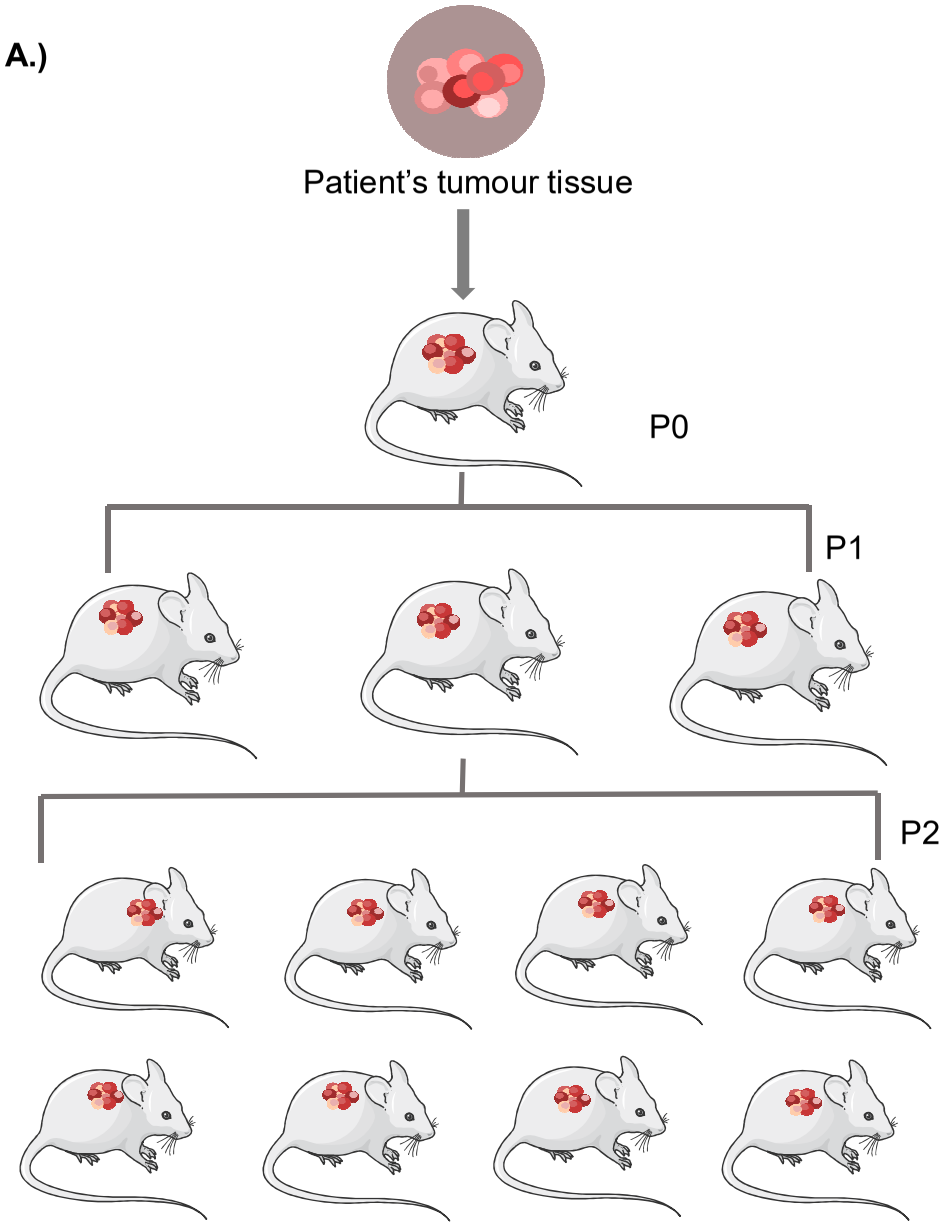
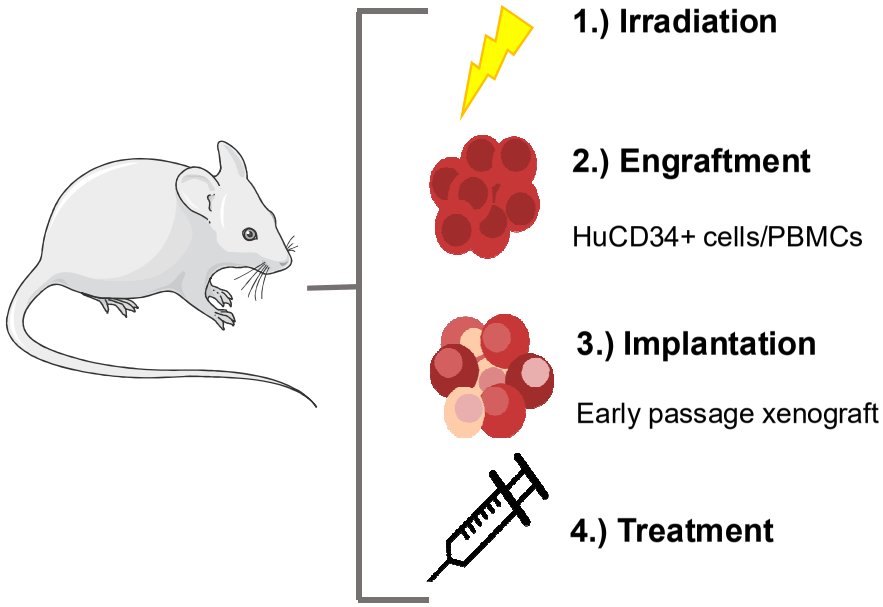


Figure 1. Generation and features of different humanised mouse models: The hu-PBL (human Peripheral Blood Leukocytes) model is generated by intravenous (IV) or intraperitoneal (IP) injection of human peripheral blood mononuclear cells (PBMCs) to an adult immunocompromised mouse. In the hu-SRC (human Stem Repopulating Cell) model, haematopoietic stem cells (HSCs) derived from either umbilical cord blood, bone marrow, fetal liver or mobilized from peripheral blood, are engrafted into irradiated adult immunocompromised mice via IV or intrafemoral (IF) injection, or into irradiated newborn immunocompromised mice via IV, intracardiac (IC) or intrahepatic (IH) injection. In the BLT (Bone marrow, Liver, Thymus) model, adult immunocompromised mice are irradiated and transplanted under the renal capsule with fetal thymus and liver fragments and injected with autologous HSCs. GVHD, graft versus host disease.


a.
b.
CD45
mCollagen
mCD31
mE-cadherin
c.
d.
hCD45


A.)
Patient's tumour tissue
P0
P1
P2


B.)
1.) Irradiation
2.) Engraftment
HuCD34+ cells/PBMCs
3.) Implantation
Early passage xenograft
4.) Treatment

Humanised Mouse Platforms			
Common abbreviation	**Material Source**	**Summary**	**Key references**
Hu-PBL	human **P**eripheral **B**lood **L**ymphocytes	Simple and cost-efficient method driven by mainly effector memory T cell engraftment.	King et al. 2008
Hu-SRC	human **S**tem **R**epopulating **C**ells	Diverse human immune repopulation driven mainly by a naïve phenotype.	Ito M et al. 2002
BLT	**B**one marrow, **L**iver, **T**hymus	Systemic repopulation of multiple lineages and superior formation of secondary lymphoid organs.	Lan P et al. 2006

Common Immunodeficient platforms				
Common abbreviation	**Genetic Background**	**The Jackson Lab Code**	**Applications**	**Key references**
NSG (**N**OD.**S**cid.**G**amma)	NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ	005557	IL-2rγ not expressed and will not bind cytokines. Supports human immune reconstitution.	Shultz, Lyons et al. 2005
NOG (**N**OD.**G**amma)	NODShi.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}	-	IL-2rγ expressed but will not bind cytokines. Supports human immune reconstitution.	Ito M et al. 2002

NRG (**N**OD.**R**ag.**G**amma)	NOD-Rag1^{null} IL2rgamma^{null}	007799	IL-2rγ not expressed and will not bind cytokines. Supports human immune reconstitution.	Pearson, Shultz et al. 2008
BRG (**B**ALB/c.**R**ag.**G**amma)	BALB/cA-Rag2^{null}Il2rγ^{null}	-	Supports human immune reconstitution.	Traggiai, Chicha et al. 2004
NSG Strains				
NSG-SGM3	NOD.*Cg-Prkdc^{scid}Il2rg^{tm1Wjl}*Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ	013062	Supports myeloid compartment engraftment, increased CD4 T-Reg cells.	Wunderlich, Chou et al. 2010
NSG-Tg(Hu-SCF)	NOD.Cg-*Prkdcscid Il2rgtm1Wjl* Tg(PGK1-KITLG*220)441Daw	-	Supports HSC engraftment without irradiation.	Brehm, Racki et al. 2012
NSG-HLA-A2.1	NOD.Cg-*Mcph1$^{Tg(HLA-A2.1)1Enge}$Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ*	009617	HLA-restricted immune responses.	Strowig, Gurer et al. 2009
NSG-HLA-A2/HHD	NOD.Cg-*Prkdc^{scid} Il2rg^{tm1Wjl}* Tg(HLA-A/H2-D/B2M)1Dvs/SzJ	014570	HLA-restricted immune responses. Enhanced humoral response.	Shultz, Saito et al. 2010
NSG-DR1 KO	NOD.Cg-Tg(HLA-DRA*0101,HLA-DRB1*0101) 1Dmz *Prkdc^{scid} Il2rg^{tm1Wjl}*/GckRolyJ	012479	HLA-restricted immune responses.	Find reference

NSG-DR4 KO	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} *H2-Ab1*^{tm1Doi} Tg(HLA-DRB1)31Dmz/SzJ	017637	HLA-restricted immune responses.	Covassin, Laning et al. 2011
NSG-B2m	NOD.Cg-*B2m*^{tm1Unc} *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ	010636	Delayed GvHD.	King, Covassin et al. 2009
NSG-(KbDb)null	NOD.Cg-*Prkdc*^{scid} *H2-K1*^{tm1Bpe} *H2-D1*^{tm1Bpe} *Il2rg*^{tm1Wjl}/SzJ	023848	Delayed GvHD.	Covassin, Jangalwe et al. 2013
MHC I/II KO	NOD.Cg-*Prkdc*^{scid} *H2-Ab1*^{em1Mvw} *H2-K1*^{tm1Bpe}*H2-D1*^{tm1Bpe} *Il2rg*^{tm1Wjl}/SzJ	025216	Delayed GvHD.	Brehm, Kenney et al. 2019
New NSG strains				
NSG-IL15	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(IL15)1Sz/SzJ	030890	Supports NK cell survival.	Brehm, Aryee et al. 2018
NBSGW	NOD.Cg.*Kit*$^{W-41J}$*Tyr*$^{+}$*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/ThomJ	026622	Supports HSC engraftment without irradiation.	McIntosh, Brown et al. 2015

NSG-PiZ	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}Tg(SERPINA1*E342K)#Slcw/SzJ	028842	Supports human and allogeneic mouse hepatocytes engraftment .	Borel, Tang et al. 2017
Other immunodeficient strains				
SRG-15	S$^{h/m}$Rag2$^{-/-}$/IL2rγ$^{-/-}$-15$^{h/m}$	-	Supports myeloid and NK cell survival.	Herndler-Brandstetter, D., et al. 2017
MISTRG	M-CSFh/h IL-3/GM-CSFh/h SIRPAh/m TPOh/h Rag2$^{-/-}$ Il2rg$^{-/-}$	-	Supports NK cell survival.	Rongvaux, A., et al 2014
HUMAMICE	HLA-A2$^{+/+}$/DR1$^{+/+}$/H-2-β$_2$m$^{-/-}$/IAβ$^{-/-}$/Rag2$^{-/-}$/IL2rγ$^{-/-}$/Perf$^{-/-}$	-	Supports HLA restricted immune reconstitution. GVHD delayed.	Zeng, Y., et al. 2017

	Flow cytometry	Mass cytometry		IHC	IMC
Measurement	Fluorescent probes	Stable metal isotopes		Chromogenic detection Fluorescent detection	Stable metal isotopes
Parameters	Up to 28	Up to 50		Multiplexing: Up to 3 for chromogenic detection Up to 5 for fluorescent	Up to 37
Sample throughput	Up to 25,000 cells/second	500-1000 cells/second			
Advantages	Live cells Cell sorting ability	No spectral overlap		Compatible with histological dyes	Multiplex over 50 markers in a single tissue . Better quantification
Disadvantages	Spectral overlap	Can not recover viable cells No cell sorting ability		Multiplexing limited	Few antibodies validated
Platform Cost	Under 500K	Significantly over 500K			Significantly over 500K