Early Immunogenicity Risk Mitigation

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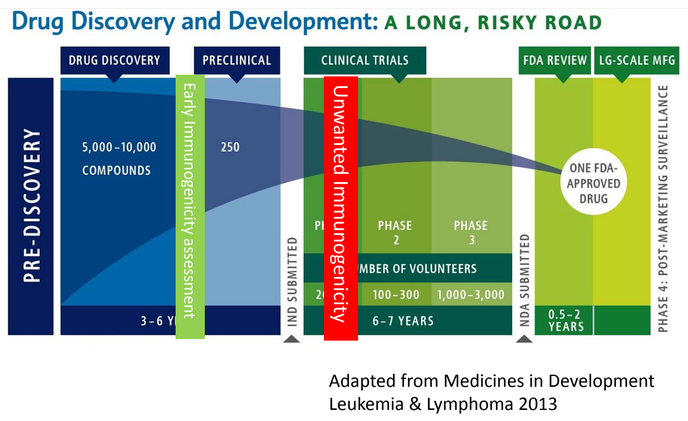
Introduction

The ability of a drug candidate to induce an unwanted immune response can be a significant hurdle during drug development and a reason for product failures. Unwanted immunogenicity can cause loss of efficacy, altered pharmacodynamics and stability problems.



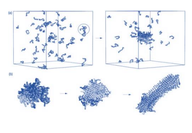
Adapted from Medicines in Development Leukemia & Lymphoma 2013

Causes of unwanted immunogenicity

Unwanted immune responses to biologics can be induced by a combination of different factors. Immunogenicity is the result of the interplay between product-, treatment- and patient-related factors.
Most of these single factors can be measured or studied using analytical tools but will not provide information on the potential impact on an immune response when administered in humans.

Examples of Product related factors

T and B cell epitopes



aggregates

formulation

Examples of treatment related factors

Dose

route of administration

length of treatment

Examples of patient related factors

HLA type
Disease status
Gender
Age

Other unknown factors

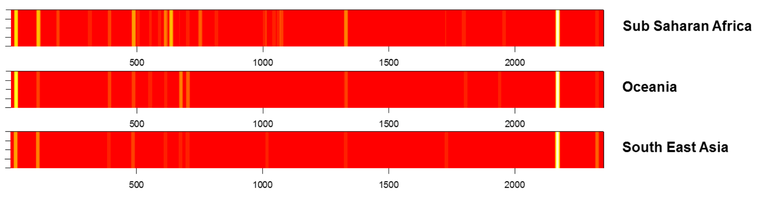
Early immunogenicity assessment

Although it is difficult to precisely predict the clinical outcome in a non-clinical setting, incorporating the assessment of the relative immunogenic potential of drug candidates early in the development phase can significantly minimize the risk later on.

In silico and In Vitro immunogenicity screening tools can be of help in assessing the immunogenic potential of drug candidates early on and are useful to rank the pipeline candidates. In the case of biosimilars, the immunogenic potential of the originator and biosimilar molecules can be compared.

In silico immunogenicity assessment using PAN-specific T cell epitope mapping

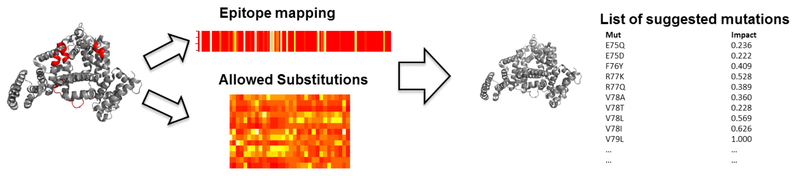
What constitutes a profound immune response is still not completely understood. However, it is well established that binding of one or more protein derived peptides to the human HLA molecule is necessary to obtain a profound adaptive immune response. The binding of peptides to an HLA molecule can be tested by various experimental methods. However the process is costly and cannot be carried out exhaustively for all HLA molecules prevalent in a given population. By using pan-specific computer based methods, a large number of MHC molecules can be included in the pre-clinical analysis and all potential risk factors identified.



Different populations identify different Th-cell epitopes. Epitopes were identified using the pan specific mapping tool NetMHCIIpan and weighted according to the frequency in each population. In total > 100 different HLA molecules were used in the analysis

In silico de-immunization to reduce/avoid unwanted immunogenicity

In-silico based T-cell epitope mapping enables multiple virtual mutations to be tested for their ability to remove epitopes. To predict the functional impact of the introduced mutations, we calculate a panel of features related to structure and function (predicted secondary structure, sequence conservation and Blosum scores) for the wild type and mutated protein sequences and only mutations not altering these features are introduced.



Conceptual drawing of the presented DeImmunization Algorithm. First epitopes are identified in the native protein sequence. Next, allowed substitutions in these areas are identified and tested for their ability to remove the epitopes.

In vitro Immunogenicity Assessment

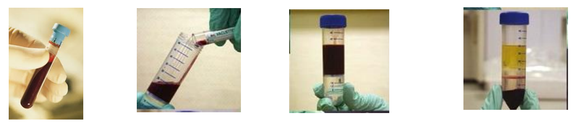
In order to mimic the human immune system as accurate as possible, immune cells from human healthy or diseased donors can be sampled and cryopreserved for in vitro testing. Peripheral Blood Mononuclear Cells (PBMCs) can be isolated from human blood.

When using optimized protocols and controlled conditions for blood sampling, shipment and PBMC isolation and cryopreservation, optimal viability and functionality of the cells will be remained.

A cohort of 50 donors is used to represent the population of interest (based on the HLA DRB1 expression and frequency in the reference population).

PBMC isolation and cryopreservation

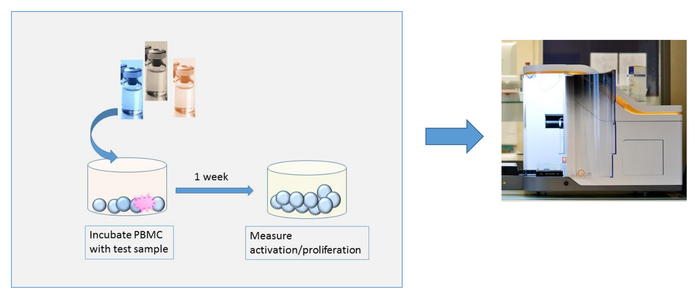
Peripheral Blood Mononuclear Cells (PBMCs) are isolated from fresh whole blood using density gradient centrifugation. After isolation and counting, PBMCs are cryopreserved using controlled rate freezing and stored in vapor phase storage tanks. After a stringent quality control, cells are used for in vitro immunogenicity assays.



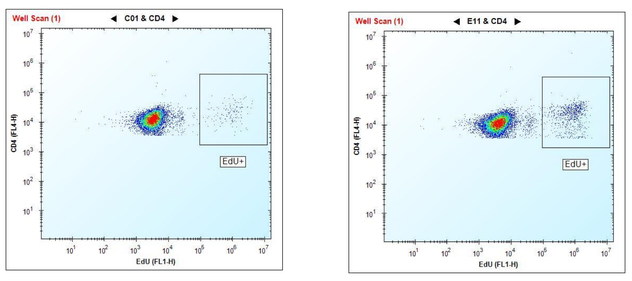
Overview PBMC isolation via density gradient centrifugation

In vitro T cell assays

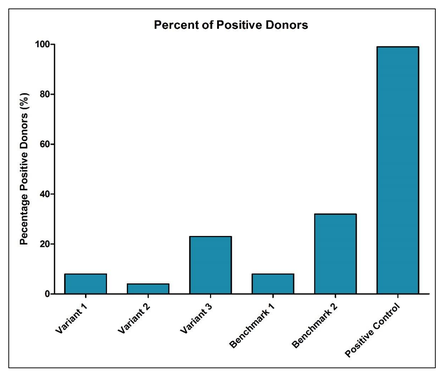
Upon the induction of an immunogenic response, helper T cells become activated and start to proliferate and produce cytokines and will assist in stimulating B cells to make antibodies.
An In Vitro proliferation assay using human PBMCs can be used to assess the immunogenic potential of lead candidates.



Schematic overview In Vitro T cell proliferation assay. Proliferation is measured using incorporation of Edu and analysed using the Intellicyt iQue Screener



Dot plot showing proliferating cells in an unstimulated (left) and stimulated (right) well



Graph showing the % of positive donors as measured in an In Vitro immunogenicity screening assay

Conclusions

Implementing early immunogenicity assessment strategies using tools like in silico T cell epitope prediction and in vitro T cell assays can contribute significantly to the successful outcome of drug development programs and reduce the number of product failures.

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