

IMMUNOGENICITY OR NOT OF BIOLOGICS IN THE SUBCUTANEOUS SPACE

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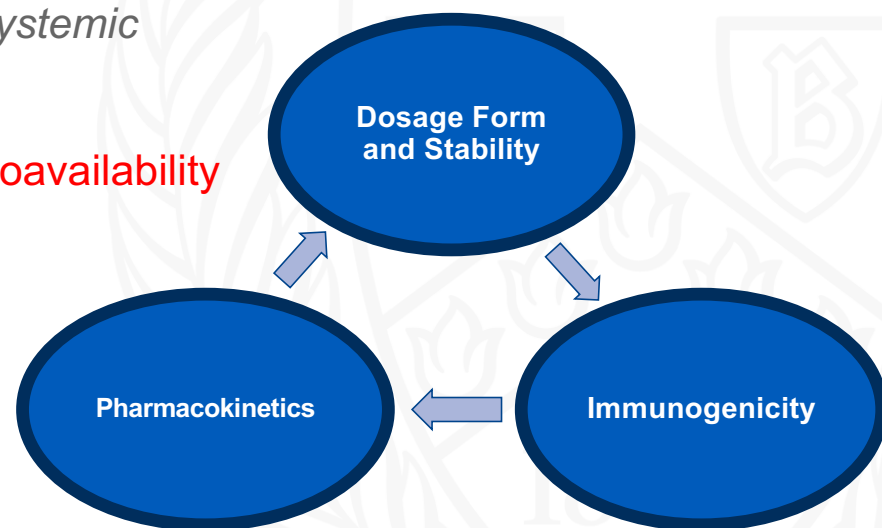
SC administration

- SC administration of therapeutics is desired for cost, convenience and compliance
- Could prolong circulation half-life due to slow absorption (*Tiede et al J Throm Haemo 2011*), tolerability to certain therapies, reduce systemic infection by iv infusion
- **Challenging** – formulation and dosage form, incomplete bioavailability and immunogenic potential

Immunogenicity – ADA, sc a treatment related factor

2/28

Turner and Balu-Iyer J Pharm Sci 2018



IV vs SC immunogenicity

Brand name (molecule)	Molecule description ⁱ	ADA incidence ^j (%)	
		SC	IV
Actemra® (tocilizumab) ^a	Anti-human IL-6R humanized IgG1κ	0.8 or 3.5	0.8 or 0
Darzalex Faspro™ (daratumumab) ^{b,c}	Anti-CD38 human IgG1κ	0	0.5
Entyvio® (vedolizumab) ^d	Anti-α4β7 integrin humanized IgG1	6	6
Herceptin®/Herceptin Hylecta™ (trastuzumab) ^{c,e}	Anti-HER2 humanized IgG1κ	16	10
Omontys® (peginesatide)	Synthetic, pegylated 21 amino acid dimeric peptide, ESA	1.9	0.7
Orencia® (abatacept)	CTLA-4 modified human IgG1 Fc fusion protein	1	2
Nucala® (mepolizumab) ^f	Anti-IL-5 humanized IgG1κ	5	4
Phesgo™ (pertuzumab + trastuzumab) ^{c,g}	Anti-HER2 humanized IgG1κ	4.8, 0.9	3, 0.4
Rituxan®/Rituxan Hycela® (rituximab) ^{c,e}	Anti-CD20 chimeric murine/human IgG1κ	2	1.9
Simponi® (golimumab) ^h	Anti-TNFα human IgG1κ	6	0



sc vs iv – sc and iv comparable immunogenicity

- However, a comparative clinical study of sc vs iv administration of abatacept, a fusion protein of Fc of human IgG and extracellular domain of CTLA-4, showed that **efficacy and immunogenicity are comparable between two routes of administration** (*Genovese et al Arthritis and Rheu 2011; Schiff Rheumatology 2013*)
- Few preclinical studies have shown that **sc route of administration does not increase immunogenicity** (*Torosontucci et al Mol Pharm 2013*)
 - For example, the relative immunogenicity of Betaseron, interferon beta, is less for sc administration compared to iv administration
- **Question: Generalization that sc route is more immunogenic than the iv route is not universally valid for all therapeutic proteins that are given in the absence of adjuvants.**

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Sc vs iv – sc space is more immunogenic

- Abatacept - could be molecule specific and long term immunogenic potential are not captured during clinical trials.
 - For example, a long-term post-clinical trial follow-up study of sc administration of adalimumab, fully human anti TNF-alpha antibody, showed that about 28% of patients eventually developed anti-adalimumab antibody that is higher than what was observed during the shorter term of the clinical trials ([Bartelds et al JAMA 2011](#))
- There are several preclinical and clinical observation that support **SC is more immunogenic**.
- A comparative immunogenicity study of three brands of insulin in type I diabetics showed an increase in incident of anti-insulin titer development, across brands, in patients self-administering via sc route as compared to iv administration in hospital in the same cohort ([Mianowska et al Pediatric Diab 2011](#))
- The sc administration of FVIII showed significantly higher total antibody titers compared to Hemophilia A mice that were given FVIII via iv route ([Peng et al J. Pharm Sci 2009](#))
- A similar observation has been made for other therapeutic proteins such as interferon alpha and human growth hormone ([Schellekens Discovery Med 2010](#)).

What drives immunogenicity of SC administered proteins

- A **mechanistic overview** of presentation and processing of proteins by immune cells given by different routes **is lacking**.
- **What are Primary antigen processing cells that process proteins given by sc and iv?**

IV and B-cells

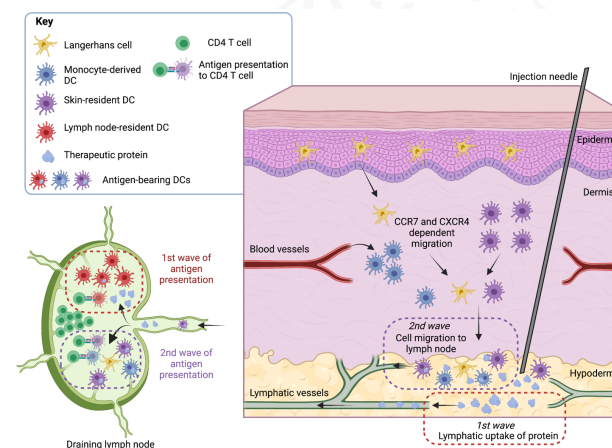
- The detection of peptide-MHC II complex using monoclonal antibodies provide an effective approach to track the fate of antigens and the cells that produce these complexes following different routes of administrations. (*Zhong et al J. exp. Med 1997; Reis E Sousa and Germain J Immun 1999, Manickasingham and Reis E Sousa J Immun 2000*)
- **iv - antigen-unspecific B-cells** rapidly take up the protein and present it in spleen within 4 hrs of administration and this is followed by presentation by dendritic cells after 24 hrs.
- Because **antigen-unspecific B-cells outnumber DCs in spleen**, the possibility of antigen specific T-cells encountering antigen presented by B-cells is much higher than dendritic cells
- **antigen unspecific B-cells** in the spleen are primary antigen presenting cells following iv administration

SC space and dendritic cells

- In contrast, **dendritic cells** present the antigen to T-cells following **sc administration**.
- Dendritic cells are primary initiator of T-cell responses
 - Dendritic cells have several receptors that can recognize, process and present it to T-cells. These receptors include C-type lectin, FcRn receptors and pattern recognizing receptors such as TLRs (*Banchereau and Steinman Nature 1998*)
 - Could be activated by some nucleic acid sequence
- SC route of administration increase the immunologic exposure of the proteins to effective antigen presenting cells such as dendritic cells
- The anatomy of sc space contributes to this exposure and in this space several dendritic cells phenotypes exist.

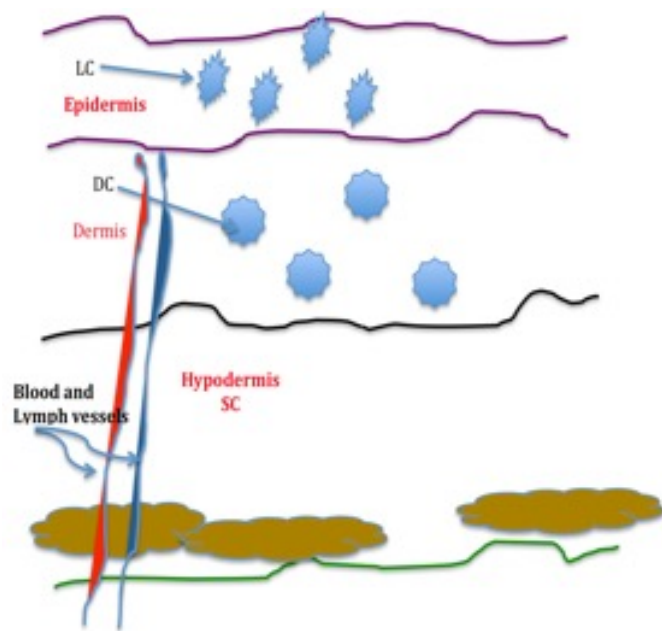
Anatomy of skin and SC space

- The most superficial layer of the skin is epidermis
- The next layer is dermis and is separated from epidermis by a membrane that supports vascular network for nutrient supply to epidermis.
- The third layer of the tissue is called hypodermis also called sc connective tissue.
- Cellular component of SC space
 - Cells – adipocytes, fibroblasts and macrophages
 - Adipocytes are found in adipose tissue lobules and fibroblasts in connective tissue septa
 - Fibroblasts – synthesize components of extracellular matrix (ECM), collagen and glycosaminoglycans and proteoglycans (hyaluronic acid), ECM is a barrier



Mirrashed et al Skin Res Tech 2004, Lanceotto, Surg 2011
 Frost Expert Opin Drug Del 2007, Bookbinder et al J Cont Rel 2007
 Fathallah et al Biopharm Drug Disp 2015; Richter et al AAPS J 2012

DCs present in skin



- Langerhans dendritic cells (LCs) reside in epidermis and dermis dendritic cells are found in the next layer.
- How protein deposited in sc space will increase exposure to dendritic cells?
- The intensity of adaptive response depends upon the transport of proteins by dendritic cells to lymphoid organs for effective presentation to T-cells.

Two waves of presentation

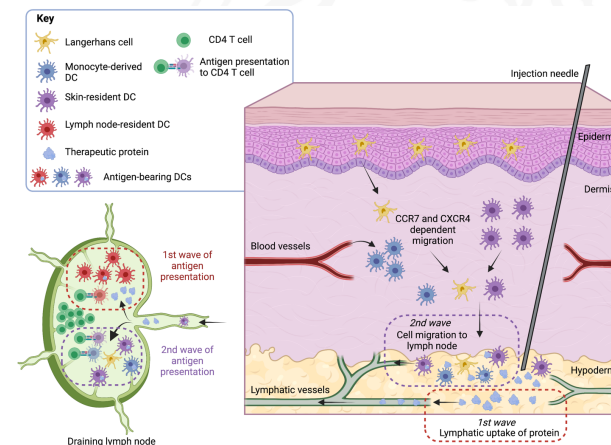
- Using peptide-MHC II complex of a fluorescent antigen and CD11c/CD40 markers, evidence of two waves of antigen processing and presentation were observed (*Ruedl et al J Immun 2000, Itano et al Immunity 2003*).

- The protein deposited in sc space is presented first by resident DCs in lymph nodes

How do proteins deposited in sc space get access to peripheral lymph nodes?

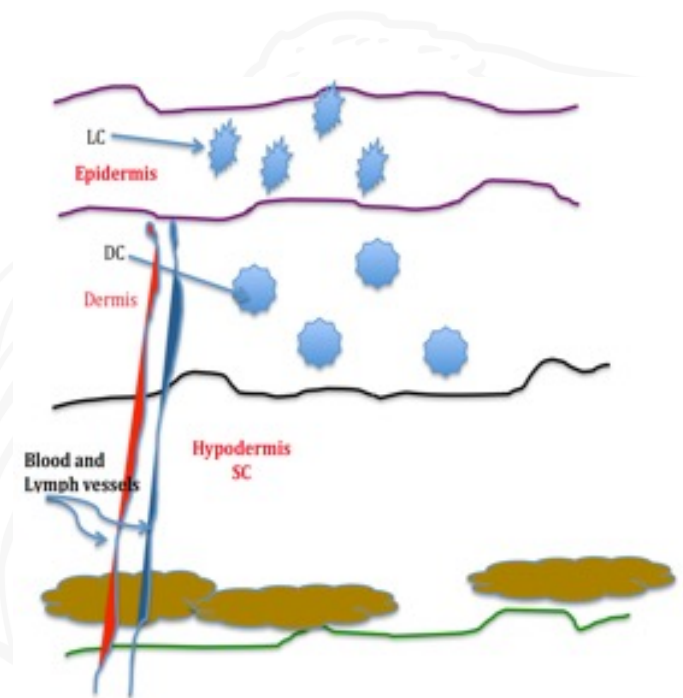
- PK studies – Lymph node distribution

- Mol wt dependent lymphatic uptake has been well established (*Porter and Charman J. Pharm Sci 2000*)



Lymph node uptake

- The pharmacokinetics and tissue distribution studies show that proteins distribute in lymph node where they can be processed by lymph node resident dendritic cells
- First wave of presentation is characterized by $CD11c^{high}/CD40^{high}$, increased E-Cadherin expression and Birbeck granule. This set of DCs arrives within hours of administration is accompanied by IL-2 production and effective proliferation of T-cells
(Reidl et al J Immun 2000, Itano et al Immunity 2003).
- **Second wave - Migration of Dendritic cells**



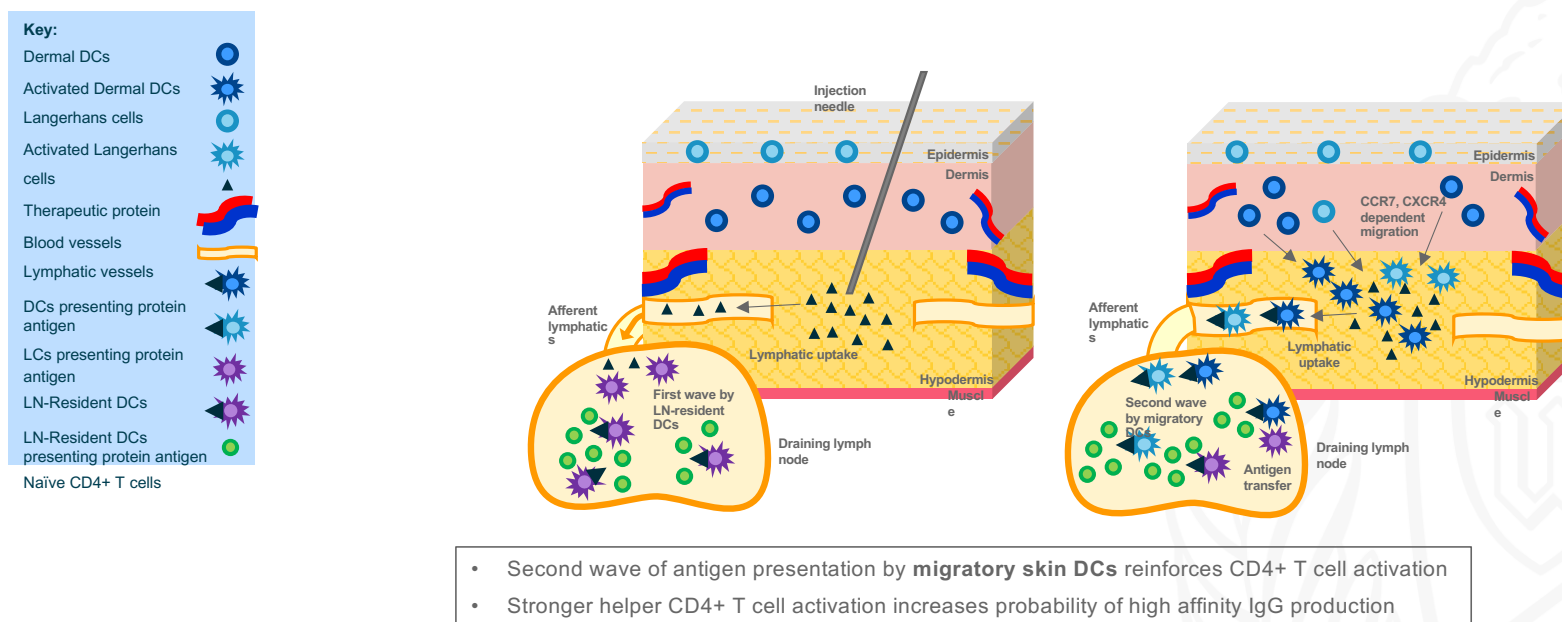
Migratory DCs

- The protein deposited in sc space trigger the uptake, processing, maturation and migration of cutaneous dendritic cells, LCs in epidermis and dermis dendritic cells to draining lymph nodes and secondary lymphoid tissues (*Hwang J Invest Derm 2012*).
- The migration of the cutaneous DCs and molecular process that drives this migration is well characterized.
- The migration of LCs is also triggered by up-regulation of two receptors CCR7 and CXCR4 (*Kabashima et al Am J Pathol 2007*)
- Triggered by inflammatory cytokines such as IL-1 beta and TNF alpha and these cytokines up-regulate VEG-F C that in turn increases the number of lymphatic vessels at inflammatory site (*Hwang J. Invest Derm 2012*)

Migration of dendritic cells

- The ligands for these receptors are expressed in lymphatic vessels and the receptor-ligand interaction drives the migration of cutaneous DCs to draining lymph nodes.
- These migratory cells display CD11c^{int}CD40^{high} and this presentation produces distinct function and is associated with a sustained expression of Il-2 receptor and delayed hypersensitivity responses.
- These migratory dendritic cells can also transfer the antigen to lymph node dendritic cells (*Allan et al Immunity 2006*)
- We propose that sc administration of the protein is immunogenic due to effective presentation of the protein to potent dendritic cells present in this space, thus increasing immunological exposure of the protein.

Two-wave mechanism of antigen presentation by dendritic cells



Utility of mechanistic understanding of SC immunogenicity

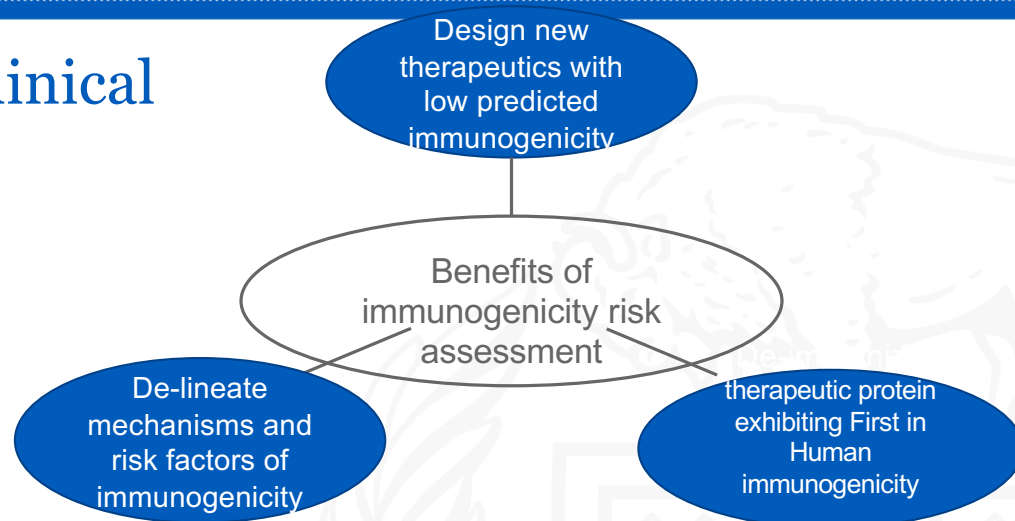
Pre-clinical Risk assessment

Rational design of mitigation strategy

Pre-clinical predictive tool for clinical immunogenicity

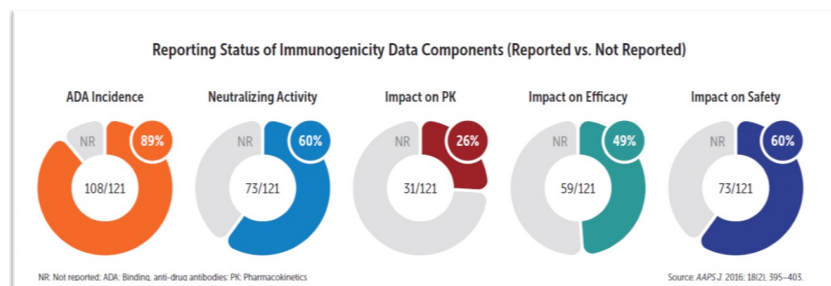
- Reduce Drug attrition and development cost

- *In silico* prediction tools
- T-epitope-MHC binding assays
- In vitro cell assays, Skin models
- Animal models



Jawa, V., et al., Frontiers in Immunology, 2020. 11(1301).

- Do not correlate with Clinical immunogenicity for several reasons



Humanization of mice



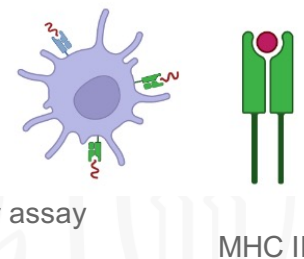
Value of Pre-clinical models



Immunogenicity risk assessment

Overall workflow:

- In silico assessment
 - T cell epitope screening/prediction using available platforms, assign immunogenicity rank/score
 - Screening protein sequence for T-cell epitopes by mapping 9-15 residue peptides for HLA binding affinity
- In vitro assays
 - HLA binding assays – test peptide epitope binding to supertype HLA alleles
 - Sensitive to quality and purity of test peptides
 - PBMC/ T cell assays
 - T-cell proliferation, cytokine analysis (ICS, ELISpot), DC/T-cell co-culture, Treg bystander assay
 - MAPPS: MHC associated peptide proteomics
 - Map potential antigenic sequences presented by APC in vitro, identify peptides presented on HLA by LC/MS

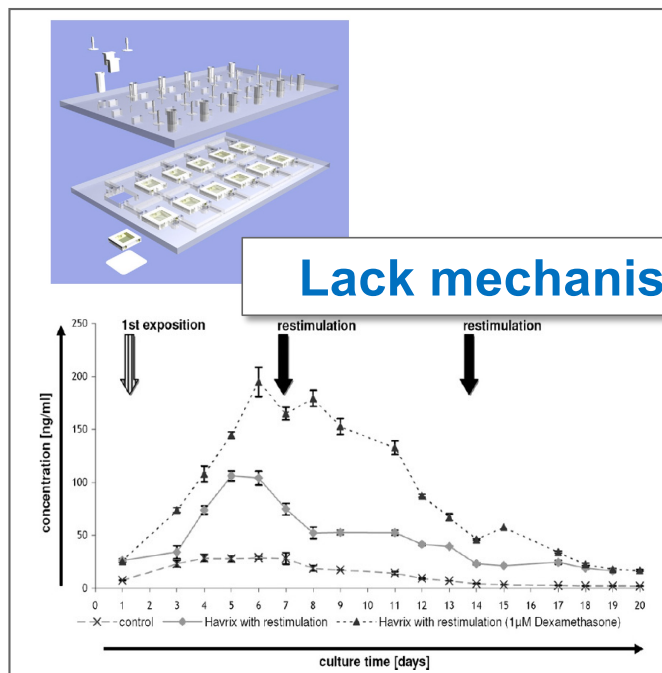


Issues with T-cell focused immunogenicity risk assessment

1. One-dimensional approach focused on late adaptive immune response
2. Requires strong, prolonged signals, i.e. long culturing time with high concentrations of therapeutic protein
3. Overlooking key steps in early immune response

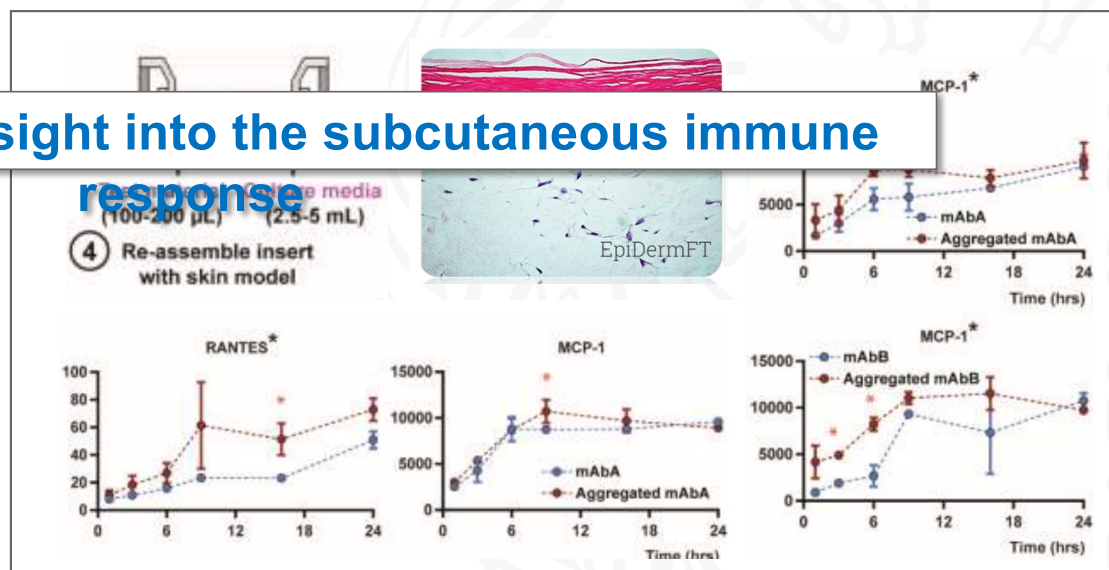
Status of immunogenicity risk assessment

- 3D lymph node model
 - Capable of IgM humoral response



- In vitro human skin model
 - Epidermis and dermis layers
 - Follow innate immune response by inflammatory mediators
 - No immune cells

Lack mechanistic insight into the subcutaneous immune response

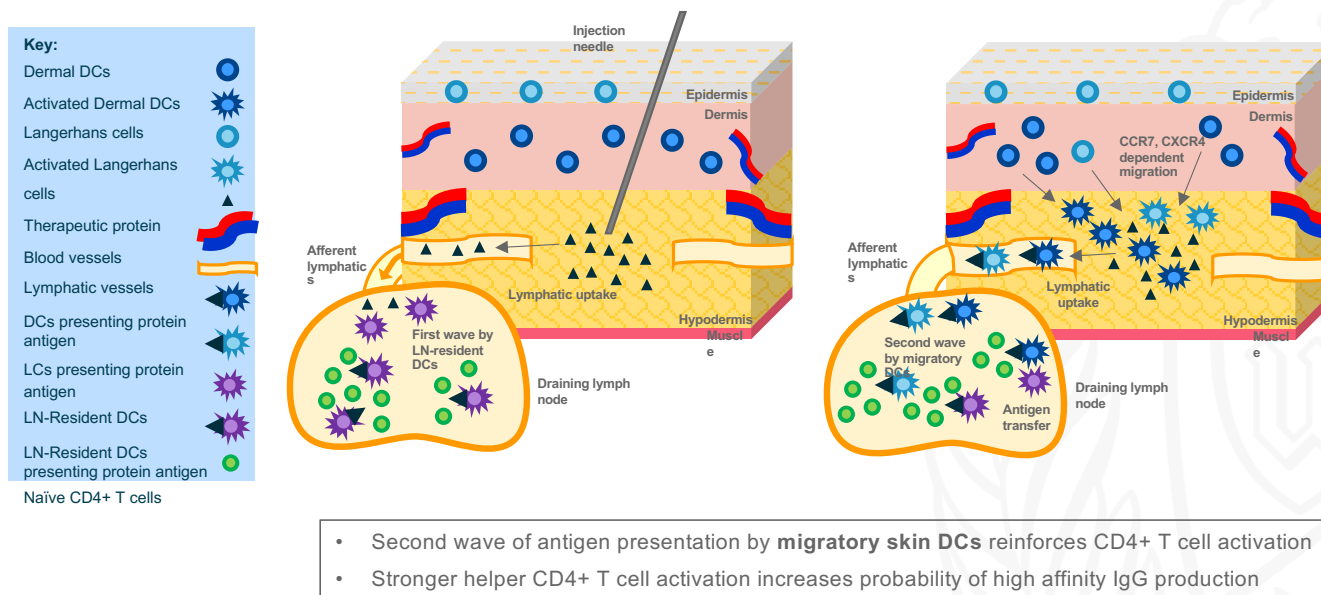


Giese, C., et al., J Biotechnol, 2010. **148**(1): p. 38-45.

Kraus, T., et al., Journal of Pharmaceutical Sciences, 2019. **108**(7): p. 2358-2366.

Tokuda, J.M., et al., Journal of Pharmaceutical Sciences, 2022.

Two-wave mechanism of antigen presentation by dendritic cells

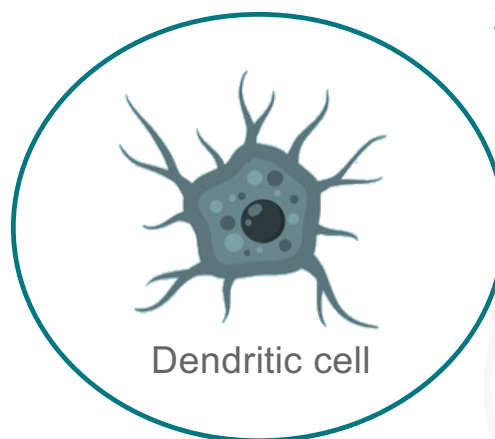


Jarvi, N.L. and S.V. Balu-Iyer, *BioDrugs*, 2021. **35**(2): p. 125-146; Fathallah, Bankert and Balu-Iyer, *AAPS J* 2013; Turner and Balu-Iyer, *JPharmSci* 2018

New mechanistic markers for immunogenicity

1. Migratory Phenotype

Expression level of chemokine receptor **CXCR4**



2. Activation Phenotype

Percent of **IL-12-producing** dendritic cells

Expression level of co-stimulatory marker **CD40**

3. Direct Migratory Potential

Number of dendritic cells migrating toward therapeutic protein in the presence of chemokines in a **Transwell** assay

Approach

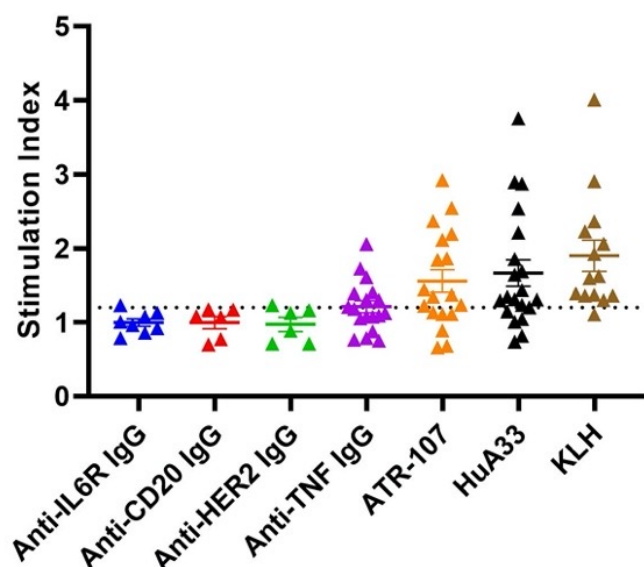
- MoDC are cultured from classical monocytes using IL-4 and GM-CSF
- MoDC are confirmed to be:
 - HLA-DR⁺DC-SIGN⁺CD11c⁺CD14^{low}
- **Immunogenicity screening panel includes:**
 - (1) IL-12, (2) CXCR4, and (3) CD40

Monocyte-derived DCs display similar phenotypic characteristics as dermal DCs – allows capturing of patient variability Grassi, F., et al., J Leukoc Biol, 1998. **64**(4): p. 484-93.

Demographic information for human PBMC donors (Cytologics LLC) included in assay validation.

Donor	Gender	Age	HLA Type
#236	Male	46	A*01:01, A*01:01; B*08:01, B*51:01; C*07:01, C*16:01/02
#919	Male	35	A*01:01, A*26:01; B*08:01, B*38:01; C*07:01/02, C*12:02/03
#589	Male	27	A*02:01, A*26:08; B*07:02, B*13:02; C*06:02, C*07:02
#773	Male	40	A*11:01, A*11:01; B*15:01, B*49:01; C*03:02/04/05, C*07:01/02/05

Assay readout

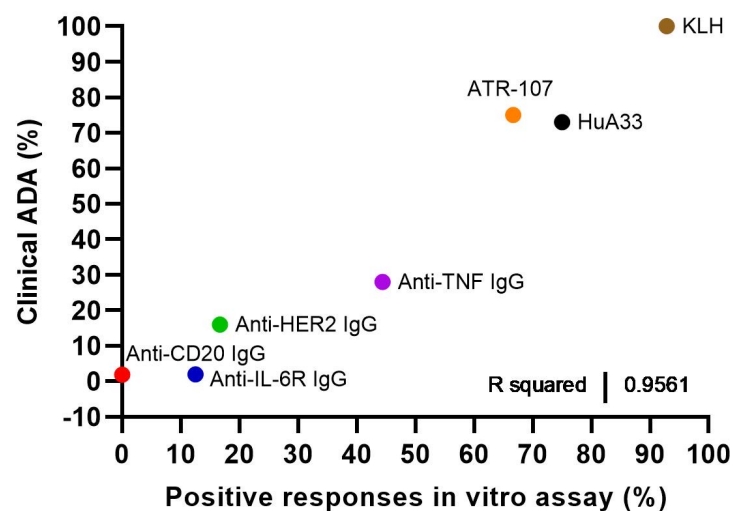


Protein	% ADA incidence
Anti IL-6R humanized antibody	2% package insert
Anti CD20 Chimeric IgG	1.9% iv and 2% sc Package insert
Anti Her2 humanized IgG	10% iv and 16% Package insert
Anti TNF-alpha human IgG	28% in RA Varies among patient populations
ATR 107 human IL-21R antibody	75% Phase 1 clinical trial
HuA33, humanized anti – A33 antibody	73% Phase 1 clinical trial
Controls OVA and Keyhole limpet hemocyanin KLH	Xenogeneic protein, induces robust T-cell response

- **Stimulation index** = fold change in marker expression over the background control (media alone)
- mAbs anti-TNF IgG, ATR-107, HuA33, and KLH (system control) have been tested in all donors
- mAbs anti-IL-6R, anti-CD20 IgG, and anti-HER2 IgG have only been tested in 2-3 donors
- A **threshold** for positive response was set at stimulation index = 1.2 which is the 95th percentile of data for anti-IL6R IgG, the low immunogenic risk control.

Jarvi and Balu-iyer 2022 Manuscript under preparation

Correlation with clinical immunogenicity



Anti-drug antibody incidence in clinical trial versus percent positive responses from *in vitro* screening assay (stimulation index > 1.2). An R-squared of 0.9561 was calculated by linear regression analysis.

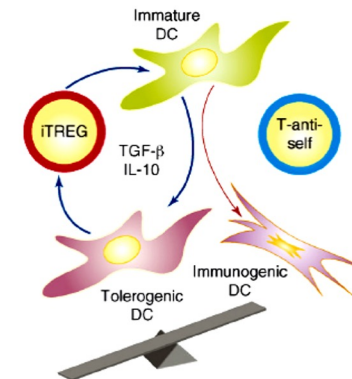
Mitigate Immunogenicity

- Mechanistic understanding could be useful in [developing rational strategies](#) to mitigate immunogenicity of therapeutic protein given via sc route.
- An important step – migration of DCs, an approach that limit the migration of DCs would be an effective step
- Inflammation triggers the migration – reduction in impurities, aggregates
- Removal of contamination – provide activation signal
- The migration is accompanied by expression of surface receptors such as CXCR4, effective formulation strategy
 - Small molecule inhibitors or antibodies against CXCR4 can also interfere with receptor binding to ligands that are expressed in lymphatics following antigen administration and this inhibition could reduce migration of DCs into the secondary lymphoid tissues.

Mitigation of immunogenicity

- The challenge of immunological exposure to dendritic cells for immunogenicity also provides an opportunity for mitigation approaches. Induction of immunological tolerance using dendritic cells is one of the effective ways to mitigate immunogenicity (*Idoyaga et al J. Clin Inves 2013*).

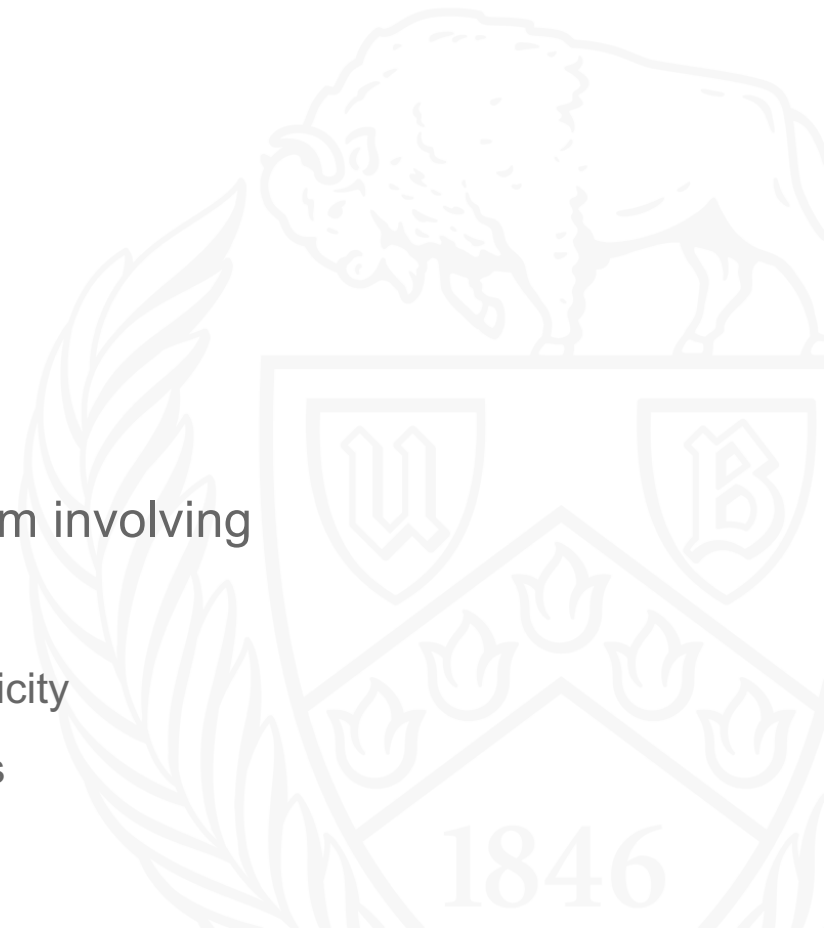
Horwitz et al Trends in Immunol 2008
Wan and Flavell 2008



- The phosphatidylserine present protein in a tolerogenic manner to dendritic cells that can promote peripheral tolerance via induction of regulatory T-cells (*Purohit et al JBC 2005*, *Ramani et al J Pharm Sci 2007*, *Gaitonde et al Clin Immun 2011*, *Gaitonde et al JBC 2013*; *Schneider et al JPharmSci 2016*, *Nguyen et al Sci Rep 2021*; *Nguyen et al JTH 2021*)

Conclusions

- SC delivery of biomolecules is challenging
 - Formulation stability and dosage forms
 - Unwanted Immune response
 - incomplete bioavailability
- Approach should consider all three pharmaceutical issues
- Mechanistic Understanding – two wave mechanism involving cutaneous dendritic cells
 - Pre-clinical Risk assessment tool to predict immunogenicity
 - Rational development of innovative Mitigation strategies



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- Several MS students

➤ Facility:

- Pharmaceutical Sciences Instrumentation Facility, Imaging and Flow cytometry facility
- Animal facility

➤ Collaborators:

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