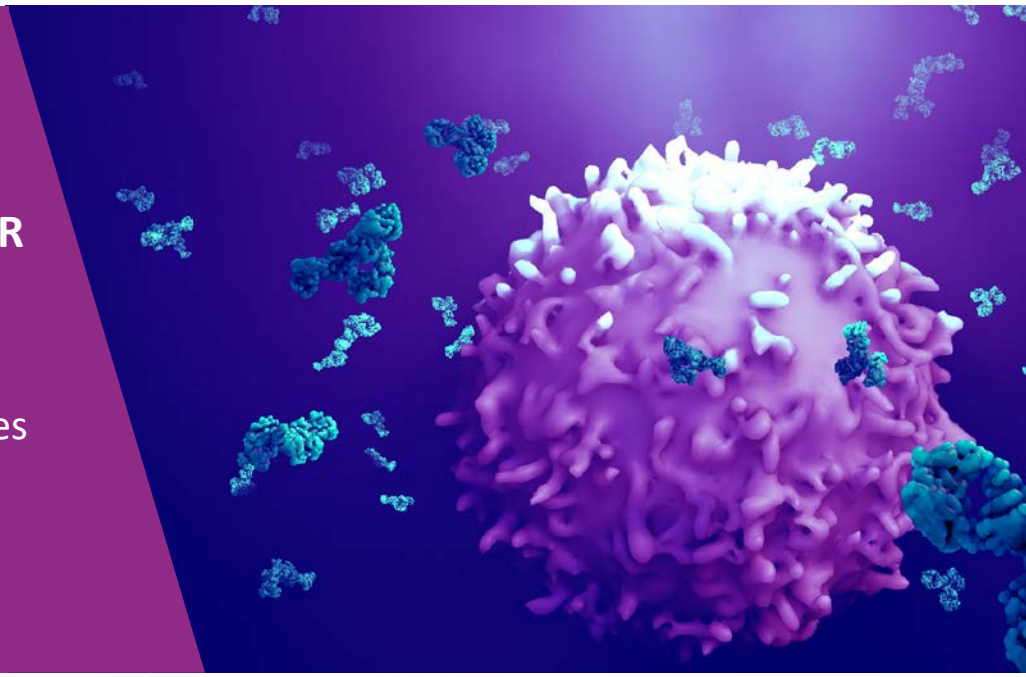
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 1.4
2nd imSAVAR Workshop

DELIVERABLE REPORT

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853988.
The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.

innovative medicines initiative

efpia

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

Abstract

The imSAVAR second stakeholder workshop was held on the fourth of November 2020. There were 51 attendees. The focus was on the Checkpoint Inhibitor Mechanism of action. The workshop began with a presentation of the work that has been done to complete a immune related adverse outcome pathway for checkpoint inhibitors.

A major outcome of the discussion was that while the original concept was that for each MoA there would be one irAOP, for checkpoint inhibitors this concept is not useful as there are multiple different types of checkpoint inhibitors with different type of toxicities and in different organ systems.

The regulatory perspective provided by one of the regulatory experts from the partner was that indeed there should be specific irAOPS. She also pointed out that there are not a lot of organ on the chip systems in the current dossiers meaning this may be something that could be a topic more formal regulatory advice.

The plan is to move forward with the different irAOPS with the development of more case studies, engagement of industry partners and evolution of the organ on a chip models.

Document Information

Deliverable Report	**D1.4: 2nd imSAVAR Workshop**
Date	**15.01.2021**
Report prepared by	**Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung e.V** **BioSci Consulting BVBA**
Project	**imSAVAR - Immune Safety Avatar: nonclinical mimicking of the immune system effects of immunomodulatory therapies** Grant Agreement No.: 853988 (IMI2-2018-15-04)
Project Coordinator	**Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung e.V.** Prof. Dr. Dr. Ulrike Köhl **Novartis Pharma AG** Dr. Jonathan Moggs
Type	**Deliverable Report | Public**

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Agenda 2nd Workshop

1. irAOP and case studies - Covance - 20 minutes
2. What do we do and what can we do - Novartis - 10 minutes
3. Discussion
    a. Who is most interested in this MoA?
    b. irAOP the right approach?
    c. Target organ specificity - which organs are the most important?
    d. Modelling autoimmune-like toxicity
4. Regulatory perspective

i. Ex. Does it make sense to do a CRS assay for this MoA?
    a. Biosimilar development
    b. Access to test molecules
    c. Awareness of other initiatives in the field
    d. Focus and plan

j. Models to work on etc.

1. Presentation on Check point inhibitor irAOP

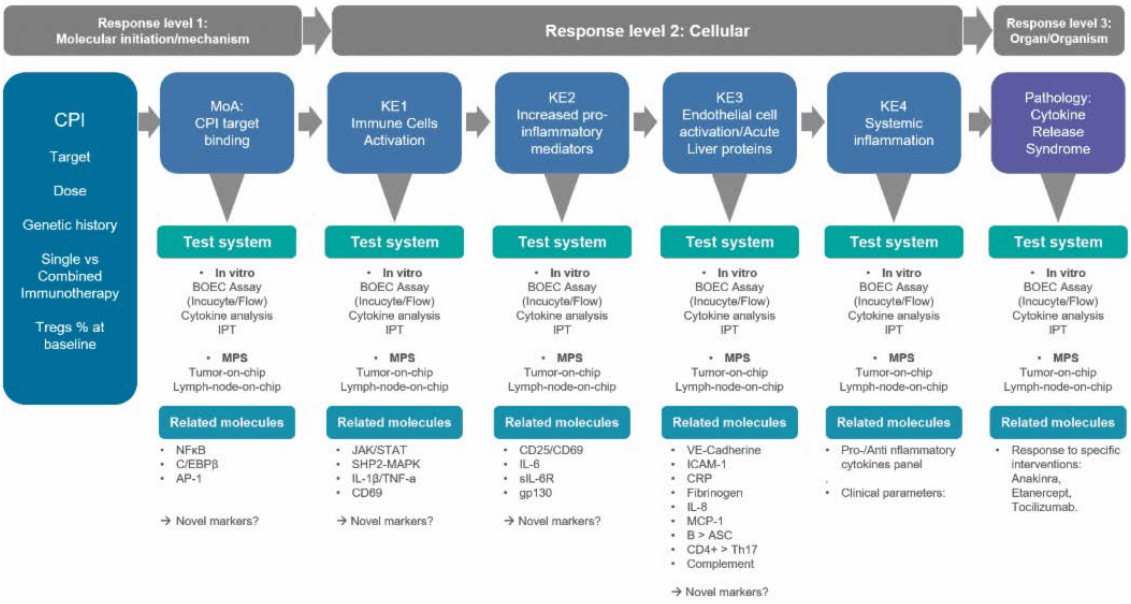
Developed based on IL-6 and Cytokine Release Syndrome because of what was found in the literature and the fact that patients respond to anti-IL6. Feedback from the consortium:

- consider combinations
- include AOPs other that CRS
- contribution of auto-antibodies
- Expand mode of action scope

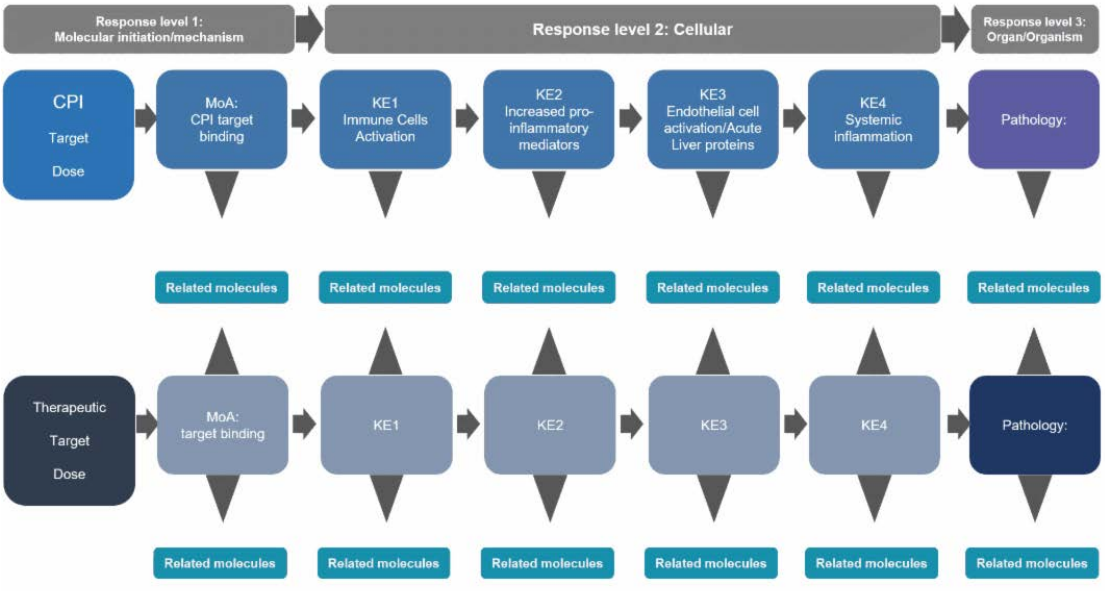
Impact

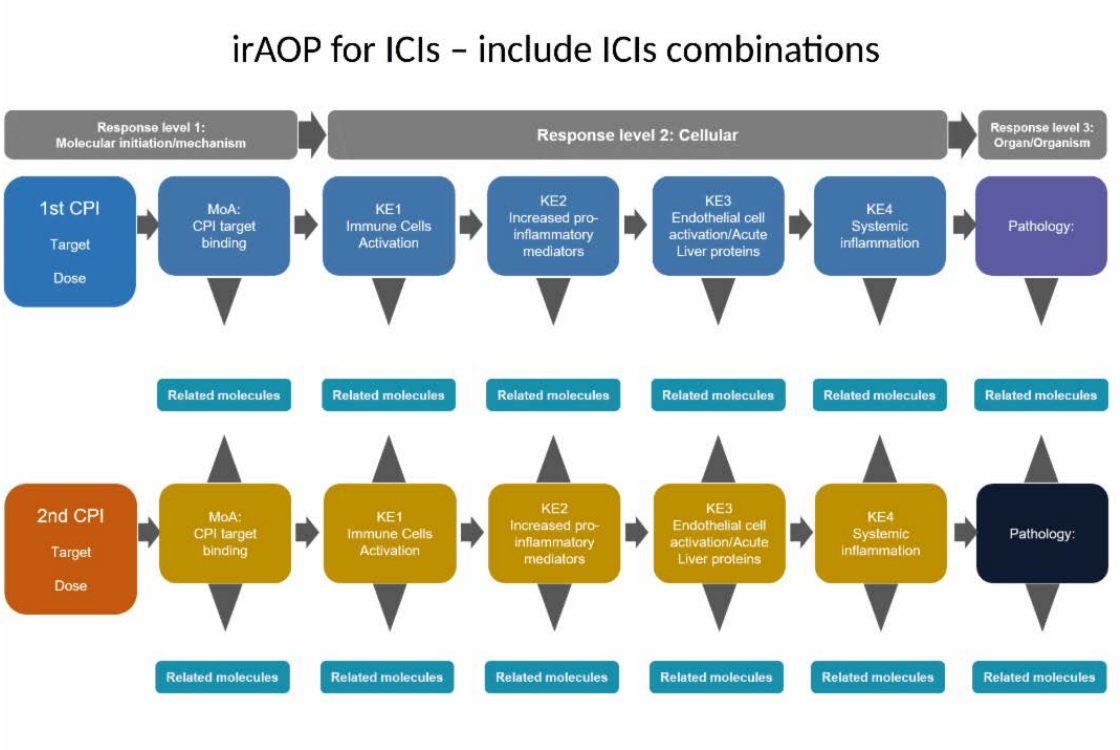
- qualify/disqualify the utiliity of the novel models/biomarkers

irAOP for ICIs



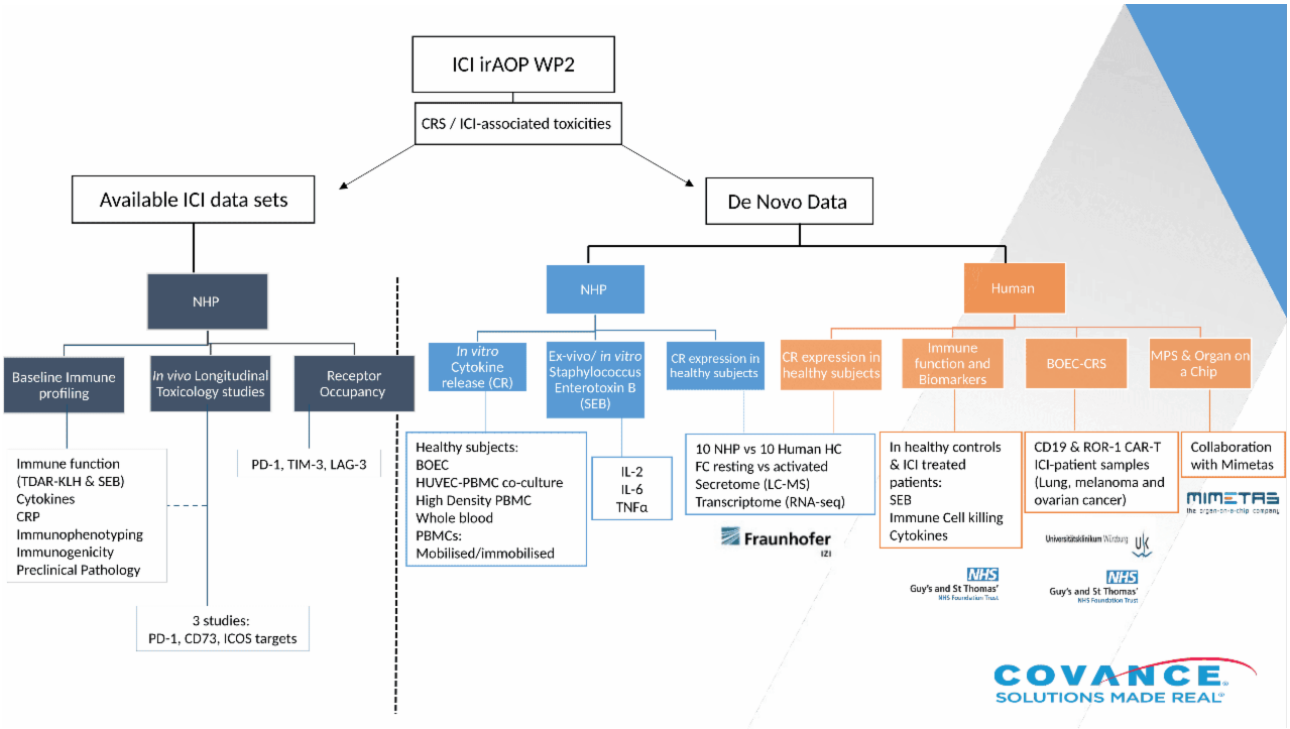
irAOP for ICIs – include ICIs combinations

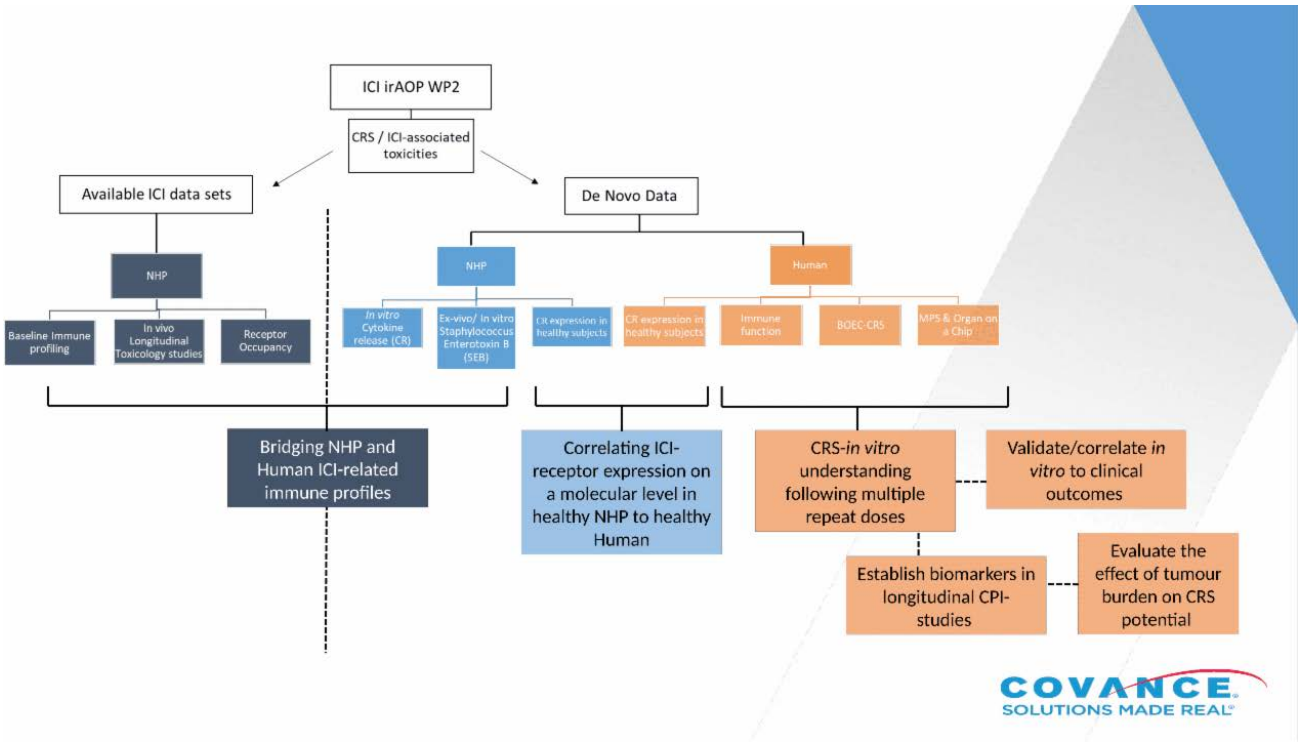




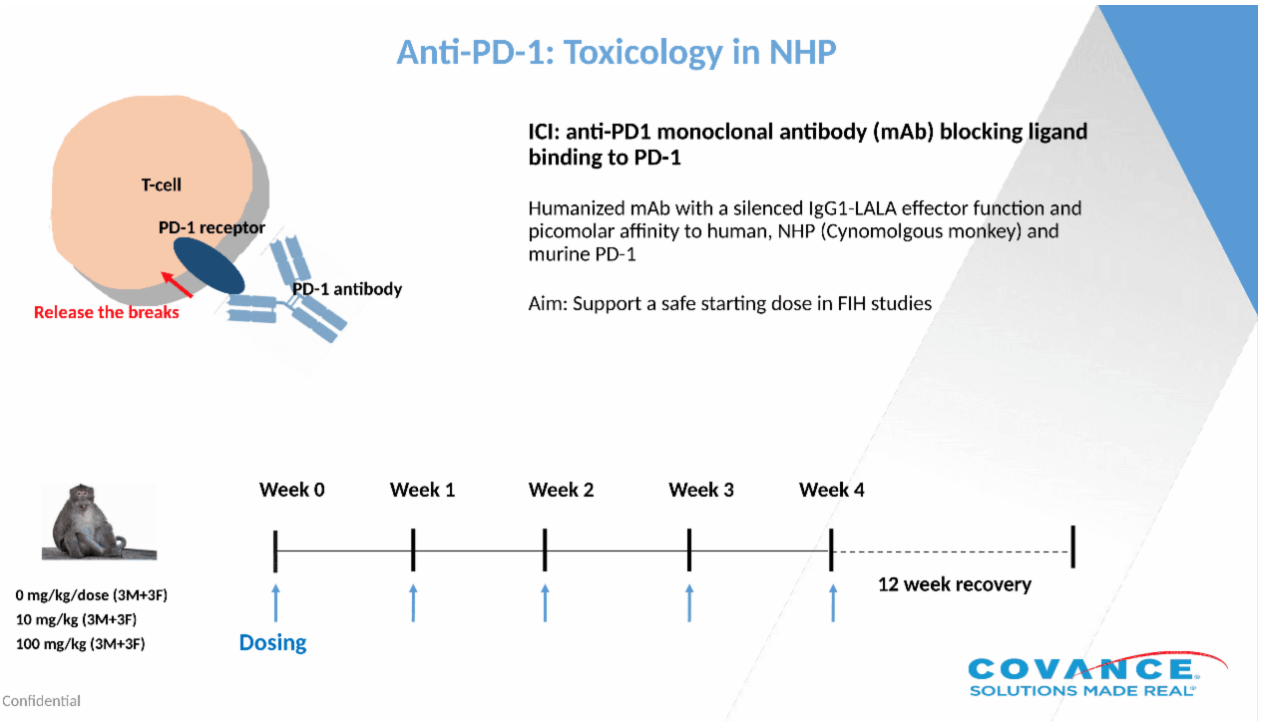
2. Partner perspectives on the CPI irAOP

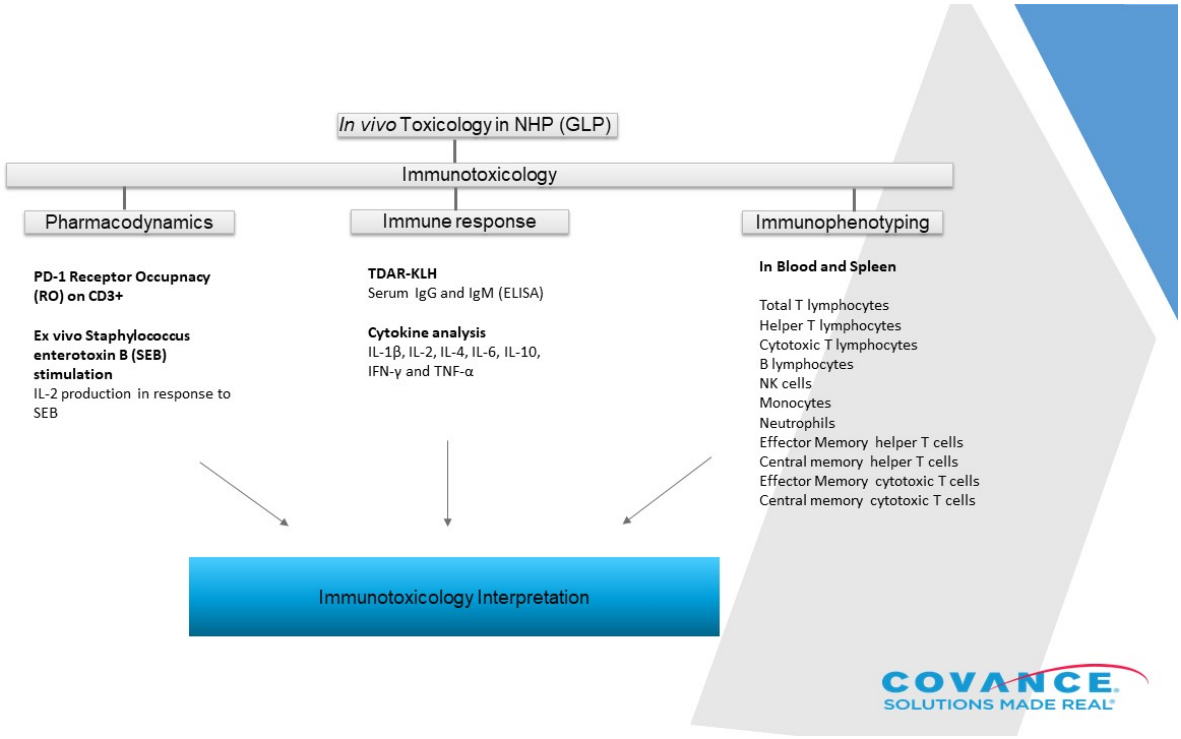
Contributions of COVANCE to imSAVAR:

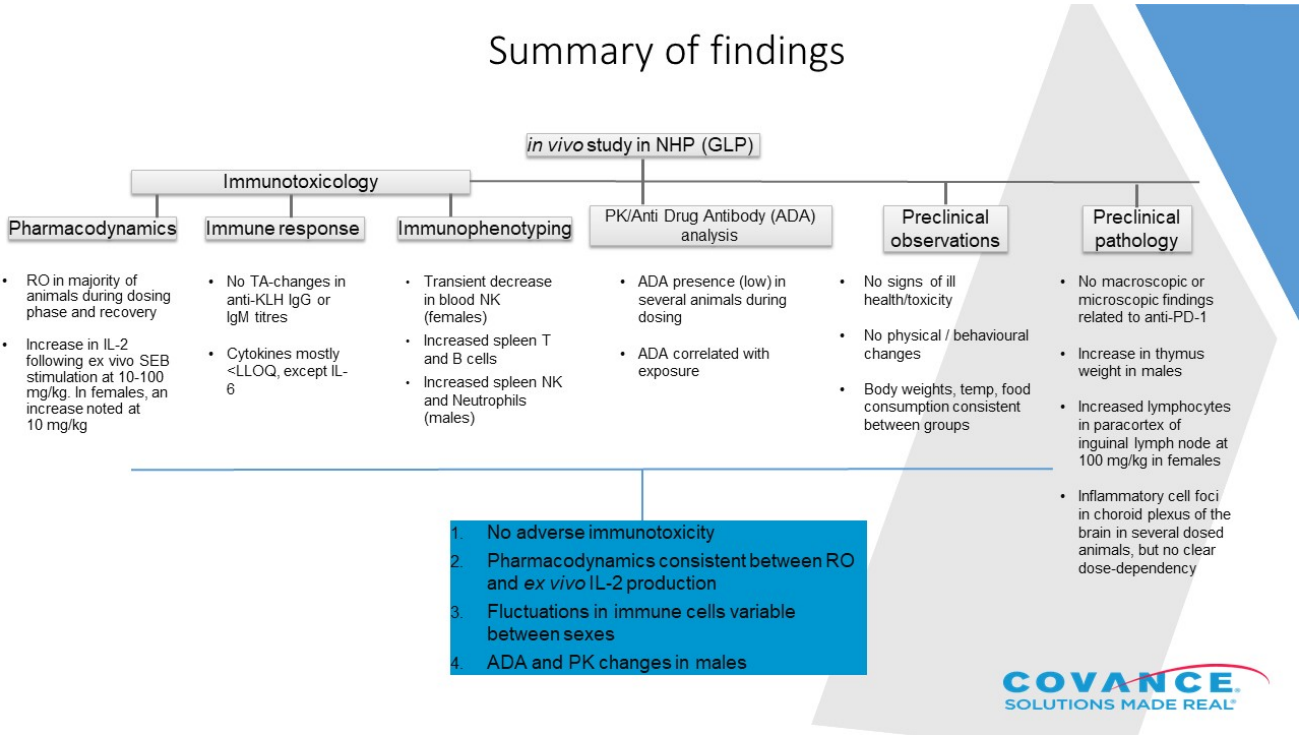




Anti-PD-1 case study



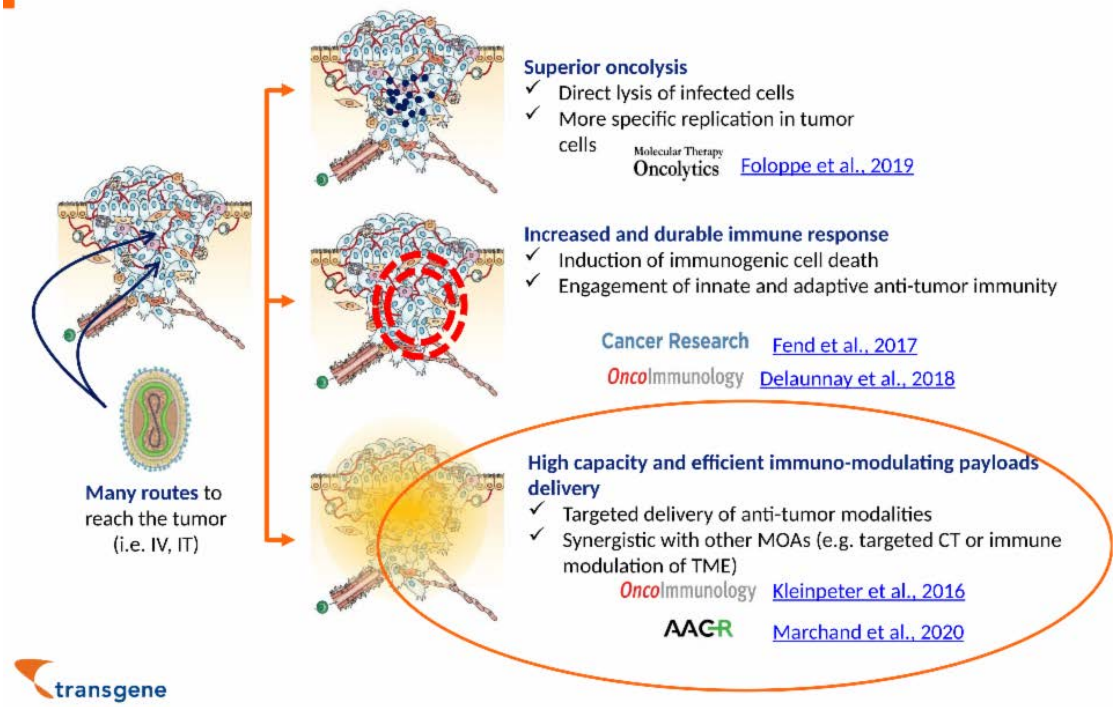




3. In Vitro Systems towards a personalised Medicine approach

Contributions of Transgene to imSAVAR:

Oncolytic Virus (OVs) & ICI : MOA



Oncolytics Vaccinia Virus (oVV) & ICI

Combination :

PDL-1: Liu, Z., Ravindranathan, R., Kalinski, P. et al. Rational combination of oncolytic vaccinia virus and PD-L1 blockade works synergistically to enhance therapeutic efficacy. Nat Commun 8, 14754 (2017).

CTLA-4: Rojas, Juan J et al. "Defining Effective Combinations of Immune Checkpoint Blockade and Oncolytic Virotherapy." *Clinical cancer Research vol. 21,24 (2015): 5543-51.*

CTLA-4 & PDL-1 : Intratumoral expression of IL-7 and IL-12 using an oncolytic virus increases systemic sensitivity to immune checkpoint blockade. Shinsuke Nakao, Yukinori Arai, Mamoru Tasaki, Midori Yamashita, Ryuji Murakami, Tatsuya Kawase, Nobuaki Amino, Motomu Nakatake, Hajime Kurosaki, Masamichi Mori, Masahiro Takeuchi and Takafumi Nakamura. *Science Translational Medicine 15 Jan 2020:Vol. 12, Issue 526.*

CTLA-4 & PD-1: *Fend L, Yamazaki T, Remy C, Fahrner C, Gantzer M, Nourtier V, Préville X, Quéméneur E, Kepp O, Adam J, Marabelle A, Pitt JM, Kroemer G, Zitvogel L. Immune Checkpoint Blockade, Immunogenic Chemotherapy or IFN-α Blockade Boost the Local and Abscopal Effects of Oncolytic Virotherapy. Cancer Res. 2017 Aug 1;77(15):4146-4157.*

Armed :

CTLA-4: BT-001, an oncolytic vaccinia virus armed with a Treg-depletion-optimized recombinant human anti-CTLA4 antibody and GM-CSF to target the tumor microenvironment (and combination with **PD-1**)

Jean-Baptiste Marchand, Monika Semmrich, Laetitia Fend, Matilda Rehn, Nathalie Silvestre, Ingrid Teige, Johann Foloppe, Linda Mårtensson, Eric Quéméneur and Björn Frendeus. *Proceedings: AACR Annual Meeting 2020; April 27-28, 2020 and June 22-24, 2020; Philadelphia, PA*

PD-1 : Vectorization in an oncolytic vaccinia virus of an antibody, a Fab and a scFv against programmed cell death -1 (PD-1) allows their intratumoral delivery and an improved tumor-growth inhibition Kleinpeter, Patricia & Fend, Laetitia & Thioudellet, Christine & Geist, Michel & Sfrontato, Nathalie & Koerper, Véronique & Fahrner, Catherine & Schmitt, Doris & Gantzer, Murielle & Remy-Ziller, Christelle & Brandely, Renée & Villeval, Dominique & Rittner, Karola & Silvestre, Nathalie & Erbs, Philippe & Zitvogel, Laurence & Quemeneur, Eric & Preville, Xavier & Marchand, Jean-Baptiste. (2016). *OncoImmunology. 5. 00-00. 10.1080/2162402X.2016.1220467.*

transgene

oVV encoding anti-CTLA-4 mAb

invir io

VV-α-CTLA-4

transgene BioInvent

- Transgene to use its Invir.IO® oncolytic virus
- BioInvent to provide full length human recombinant anti-CTLA-4 Ab

- **Improved efficacy** compared to combination of separate Ab and OV
- Longer **duration of expression**
- **Expected improved tolerability** owing to lower systemic antibody exposure in peripheral non-tumor compartments

Transgene's VV_{COP} TK-RR- can express mAbs in the tumor

OncoImmunology Kleinpeter et al., 2016

BioInvent's anti-CTLA-4 Abs promotes depletion of intratumoral Treg cells

Cancer Cell Vargas F. et al.,

Combination of ICI and oncolytic VV treatments are additive

CANCER RESEARCH Fend et al., 2017

transgene

4

oVV Virotherapy AES

Therapeutic mode of action	Models/End point	Clinical Adverse Events
Depending on OV arming **Cytokines**: Proliferation CTL activitiy Memory cross-priming, expansion of tumor specific T_H1 , reverse tolerance **ICI:** anti-tumoral activity, combinations boost effects with Ovv	**Human in vitro Models** -cell lines -patient derived Models (organoïds, 3D printed): Lung, CRC PBMCs: Functional assays (MDSC + T proliferation, M2+T proliferation, NK cytolysis assay -Skin Model **Non-Human primates** Skin lesions, viral shedding, PK of transgene product **Dog Patients** **Mouse models*** Syngeneic models: IFN production, cytotoxic activity, … Nude Mice efficacy PDX mice efficacy	Flu like syndrome (fever, chills, headache, …) Skin lesion Vascular disorder: Mild to moderate hypotension (Transient) Hepatotox AST, ALT (Rare event) Heart failure (rare event) Neurotoxicity (rare event)

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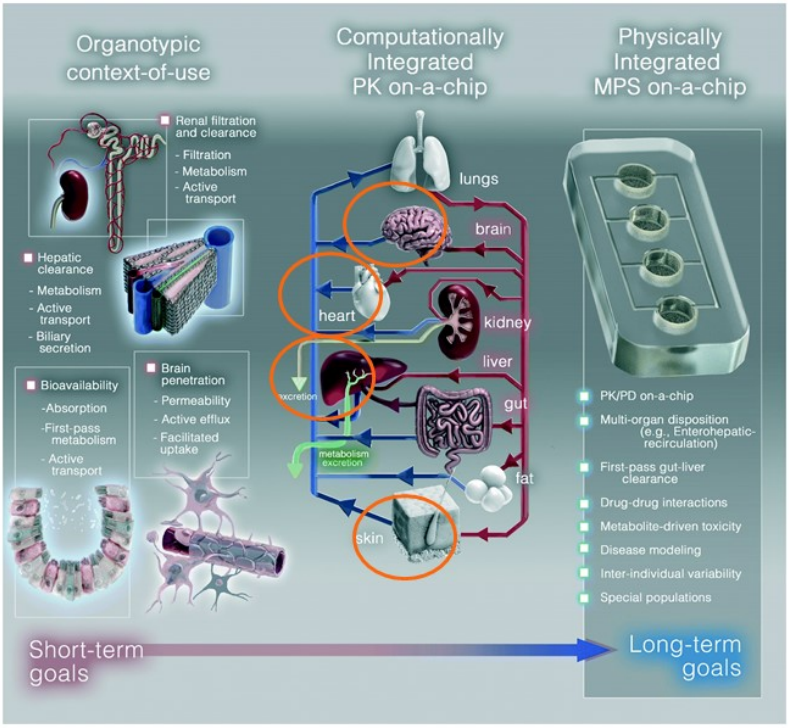
* Low permissivity to « human » OVs

oVV plus ICI combination Tox on chip

Lab Chip, 2020, 20, 446



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6

3Rs Transgene objectives

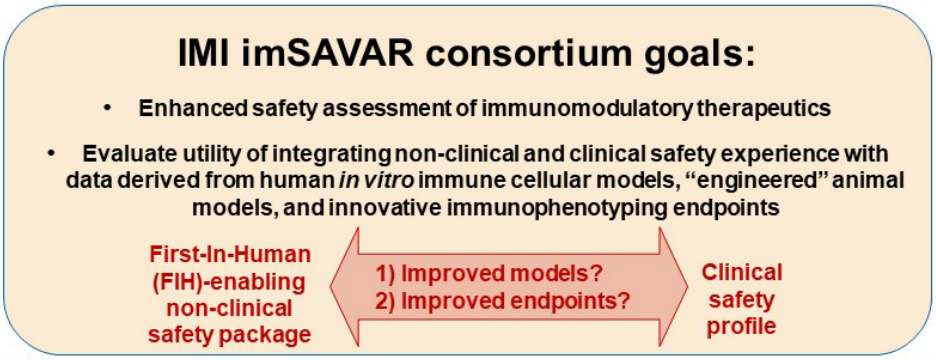
- Making **personalized organs-on-chips** from tissues of specific patients in order to select best therapeutic (High Content Low Throughput):
  - MOA
  - Tox (skin, brain, heart, liver)
- **Tissue-Tissue** Interface (Blood vessels, Brain blood barrier)
- **Human tumor** and **healthy** tissues (Gastro-intestinal models, Lung, Skin (oVV-CTLA-4), Liver Mets)
- **Endothelial** cell (Vasculature Model, Lymph circuit, …)
- **Immune** compartment (Synthetic LN, PBMC, …); Immune cells trafficking in presence of tumor cells compartment

transgene

4. What do we do and what can we do?

Jonathan Moggs (Novartis):

- There are significant clinical toxicities in checkpoint inhibitors
  - Hepatotoxicity
  - Cytokine Release syndrome
  - Cardiovascular
- Combination therapies have a synergistic toxicity
- MoA scope is very broad
- Combinations are also very broad
  - What is the best way to assess combination immune toxicities
- There are lots of clinical databases
- Drug inserts are great sources of list of toxicities seen
- Should be focused on more severe and difficult to predict toxicities that are dose limiting
- Anticipated impact
  - Benchmark models/biomarkers in animal models
  - Identify safe first in human start dose
  - know how to monitor in clinic
  - identify mitigation strategy
  - Biosimilars need nonclinical models to assess that thet are not
- anti-CTL4 case study as presented by Jim Munroe
- Can't do too many toxicities or MoAs
  - Liver Toxicity looks the most promising



Initial focus on therapeutic modes of action where prediction of human toxicities is suboptimal	Models/endpoints	Clinical adverse events
Treg modulators (e.g. rhIL-2) Immune checkpoint inhibitors (antibodies, small molecules; oncolytic viruses; combinations) Engineered T cell therapies (e.g. CART) T cell engaging antibodies (e.g. CD3 Bispecifics)	Human *in vitro* immune models (+/- patient-derived cells) Humanized animal models Enhanced pharmacodynamic and toxicodynamic biomarkers	Target organ-specific toxicities Autoimmune-like phenotypes Cytokine release syndrome & neurotoxicity

Enhancing safety assessment for immune checkpoint inhibitors

(antibodies, small molecules, oncolytic viruses; combinations)

Initial focus on therapeutic modes of action where prediction of human toxicities is suboptimal	Models/endpoints	Clinical adverse events
Immune checkpoint inhibitors (antibodies, small molecules; oncolytic viruses; combinations)	Human *in vitro* immune models (+/- patient-derived cells) Humanized animal models Enhanced pharmacodynamic and toxicodynamic biomarkers	Target organ-specific toxicities Autoimmune-like phenotypes Cytokine release syndrome & neurotoxicity
Immune checkpoint inhibitor combination toxicities Hepatotoxicity of immune checkpoint inhibitors: An evolving picture of risk associated with a vital class of immunotherapy agents Cardiovascular toxicity of immune checkpoint inhibitors in cancer patients: A review when cardiology meets immuno-oncology Cytokine Release Syndrome During Sequential Treatment With Immune Checkpoint Inhibitors and Kinase Inhibitors for		Hepatitis Cytokine release syndrome Fulminant myocarditis Skin toxicity

Translational safety assessment case studies for immune checkpoint inhibitor Ab combinations

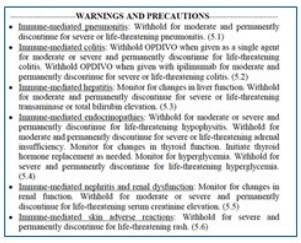
MoA Scope:

- T cell checkpoint inhibitor antibody combinations (+ biologic or LMW immunomodulators); Oncolytic virus-mediated delivery of T cell checkpoint inhibitor antibody combinations; e.g.'s anti-CTLA-4 Ab(Ipilimumab)/vermurafenib; anti-PD-1 (Pembrolizumab)/preladenant; T-cell costimulatory receptor CD137(4-11B) agonist Abs; anti-CD73; other
- Myeloid checkpoint inhibitors (e.g. SIRPa antagonist) and combination with T cell checkpoint inhibitor Abs

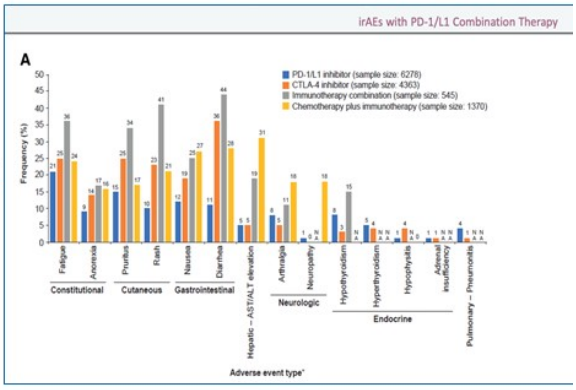
Particular emphasis on combinations that are likely to result in strong (additive or synergistic) immune stimulation:

- guided by clinical safety data
- drug labels (e.g. USPIs, EPARs)
- literature
- industry partner experience
- Incidence/severity of anticipated phenotype(s)

USPI Drug Labels for Nivolumab, Atezolizumab, Keytruda indicate typical organs affected include liver, lung, kidney, skin, GI and endocrine organs.


WARNINGS AND PRECAUTIONS

- Immune-mediated pneumonitis: Withhold for moderate and permanently discontinue for severe or life-threatening pneumonitis. (5.1)
- Immune-mediated colitis: Withhold OPDIVO when given as a single agent for moderate or severe and permanently discontinue for life-threatening colitis. Withhold OPDIVO when given with ipilimumab for moderate and permanently discontinue for severe or life-threatening colitis. (5.2)
- Immune-mediated hepatitis: Monitor for changes in liver function. Withhold for moderate and permanently discontinue for severe or life-threatening transaminase or total bilirubin elevation. (5.3)
- Immune-mediated endocrinopathies: Withhold for moderate or severe and permanently discontinue for life-threatening hypophysitis. Withhold for moderate and permanently discontinue for severe or life-threatening adrenal insufficiency. Monitor for changes in thyroid function. Initiate thyroid hormone replacement as needed. Monitor for hyperglycemia. Withhold for severe and permanently discontinue for life-threatening hyperglycemia. (5.4)
- Immune-mediated nephritis and renal dysfunction: Monitor for changes in renal function. Withhold for moderate or severe and permanently discontinue for life-threatening serum creatinine elevation. (5.5)
- Immune-mediated skin adverse reactions: Withhold for severe and permanently discontinue for life-threatening rash. (5.6)



Translational safety assessment case studies for immune checkpoint inhibitor Ab combinations

Key areas of interest:

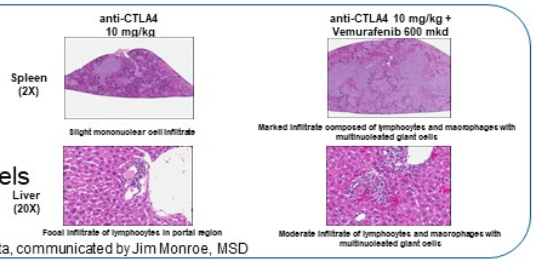
- Exploring the utility of humanized immune system rodent models and genetic manipulation of therapeutic targets to investigate mechanistic basis and pharmacodynamic/toxicologic biomarkers for clinical safety signals
- Exploring utility of human *in vitro* immune cell models for characterizing/predicting irAEs (in particular immune-mediated liver, heart and skin toxicity; "autoimmune-like" phenotypes)
- Benchmark imSAVAR models versus established *in vivo* rodent/non-rodent IND/FIH-enabling non-clinical and clinical safety data.

Anticipated impact:

- to qualify/disqualify the utility of novel models/biomarkers for enhanced safety assessment; potential impact on setting safe FIH start dose and on clinical safety monitoring and mitigation strategies; potential impact on biosimilar comparability studies

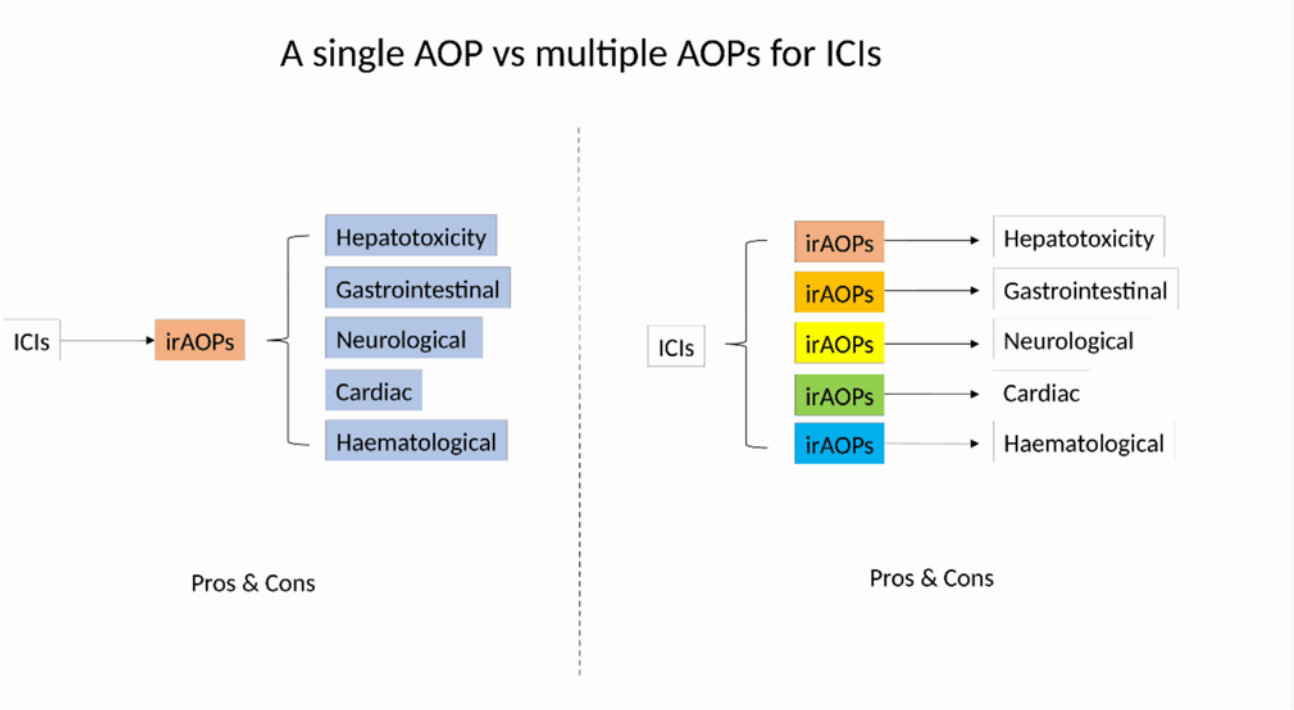
Initial experimental focus:

- Clinical hepatotoxicity associated with checkpoint inhibitor Ab/kinase inhibitor combinations
- Refine humanized immune system rodent models
- Assess feasibility of modelling in vitro?



Unpublished data, communicated by Jim Monroe, MSD

5. Should you have a single irAOP for each case study or multiple irAOPs for each case study?



- Single irAOP vs multiple irAOPs
  - Trying to write one irAOP was difficult because there are multiple different toxicities and was hard to put into one irAOP
  - Some of the toxicities will have shared elements, but the histology of different organs are different
  - When you combine therapies it will get quite complex
- Might be best to start with the most severe and dose limiting toxicity difficult to predict - then as you combine the complexity will grow
- The toxicities link well with the MPS models that are now listed in the consortium
- They would be a sort of mind map that could be used to dissect the toxicities the irAOP could be a benchmark for how to choose your models
- This will allow subteams to make the case for the individual toxicities
- In the chemical toxicity field everything starts with a molecular initiating event and often they are very similar and it is okay to use assays for the building blocks that may be used across multiple outcome pathways
- AOP is an analytical construct to explain a clinical outcome

6. Is the irAOP the right approach?

- Target organ specificity - which organs are the most important? Modelling autoimmune-like toxicity - how can this be done?
  - This is a big challenge

- In the organ on a chip you do not have a model of a artificial lymph node but there are macrophages and NK cells, but Alexander and Peter are working on an artificial lymph node

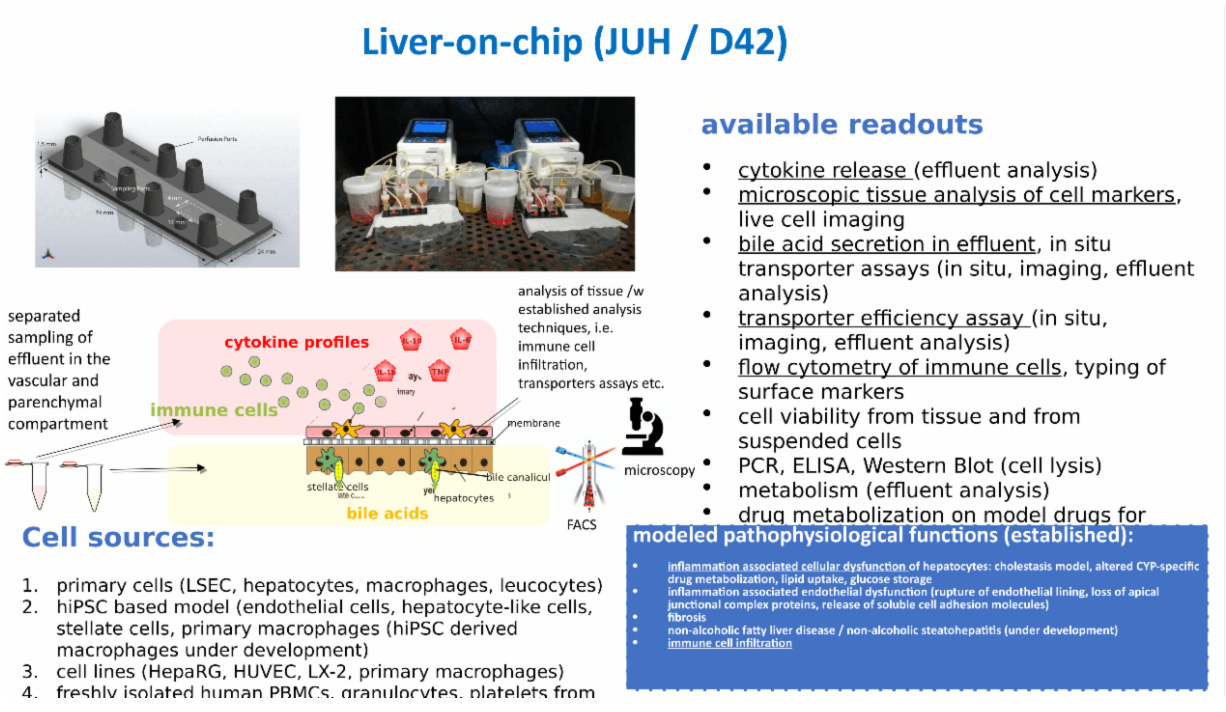
- It is really dependent upon the immune repetoire of the patient and this would make it very difficult to predict underlying immune response
- You could in the future you could incorporate cells from an individual patients.
- Should be three different phases
  - Healthy cell models to reflect complexity
  - Patient derived cells
- Patient specific models

7. Regulatory perspective

- Ex. Does it make sense to do a CRS assay for this MoA?
- Biosimilar development
- Access to test molecules
- Awareness of other initiatives in the field
- Gaby has been working at PEI on monoclonal antibodies has not come across AOPs in the documentation so far. For small molecules they would have come in contact on this
  - It might be good to join the safety assessment board and the EMEA. This is something we should pursue and Gaby has contacts with them.
  - They have also not seen a lot of organ on a chip models in the dossier's but there is interesting in learning about these techniques

8. Organ on chip models

Liver toxicity



- in vitro with multiple cell sources and multiple readouts
    - Can look at vasculature and hepatic chamber
  - Look at clinical readouts and any type of assay that can be performed in a 69 well plate that would allow for the testing of inhibitors
  - Could be a bridge between preclinical models and clinical outcomes
  - Has the model been tested with a positive control - Rilumab
- What kind of samples would you use in the system?
  - It is easy to use the primary cells of patients into the model and iPSCs
- Can you differentiate the cells from blood?
    - So, far it has been done with iPS cell line
    - Also possible to use primary lines
    - The immune response is not so high to limit the use when it is not autologous
  - If there are protocols available to derive ipsc's from PBMCs then it would be able
- What physiological conditions are being used?
  - Model tested for TLR agonists for inflammation and sepsis
- There are at least organ on a chip for both cardiovascular and skin
- Organ on the chip need to be benchmarked against clinical outcomes is it a better models to predict outcomes.
  - Once you establish you irAOPs and their blocks the first step would be to run the models and see if you can predict toxicities in a known case study
  - irAOPs can also be used to track progress you may also find that the seqeunce of key events
- Broad biomarker screening is difficult with bridging to the patient to be able to select which cytokines you have to measure in patients which fits with the work that is ongoing helps in this and how do you use that information
  - For predicting you must know the pathways in patients and linking to immunobiologstis as well

9. Who is most interested in this MoA?

- Covance
- Transgene
- Novartis
- several companies have assets that are checkpoint inhibitors. We should survey them. If it is a very few partners we have to ask if it is worth putting resource to this development.
- HESI has had working groups and public domain and most events were on the checkpoint Abs themselves. We need to make sure that we have a sense of the field at large.

10. Plan for moving forward

1. Survey industry and external groups for interest in MoA
2. Build case studies to add to the three you have
3. Develop toxicity specific irAOPS
4. Reach out to the safety committee EMEA
5. Bring biomarkers from WP 4 - linking to clinical and other MoA's

6. Test models in irAOPS to predict toxicities in the case studies
7. Develop organ on chip models
8. Adjust irAOPS

Acknowledgement

The authors would like to express their gratitude to the Innovative Medicines Initiative 2 Joint Undertaking (JU) for the financial support of this research under grant agreement No 853988. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.