Department for International Trade: Immuno-oncology event, Madrid.

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“Factors to consider when running in vivo immu oncology studies.”

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Contents

- A brief overview of Axis Bioservices
- Data requirements ahead of study initiation
- In vivo models for your compound
- Syngeneic models: setup & learnings
- Immune markers: approaches/targeting
- FACS analysis
- Case study
- Concluding thoughts
- Northern Ireland!

Axis Bioservices

27 employees running fee for service client projects.

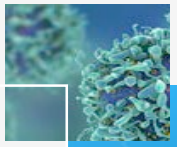
Axis Bio: Locatio

Located on the No
Northern Ireland.

Possibly familiar
Thrones filming l
the Giant's Cause

Land border with
EU…..another pre
topic in itself….

Therapeutic focus: oncology under pinned by FACS



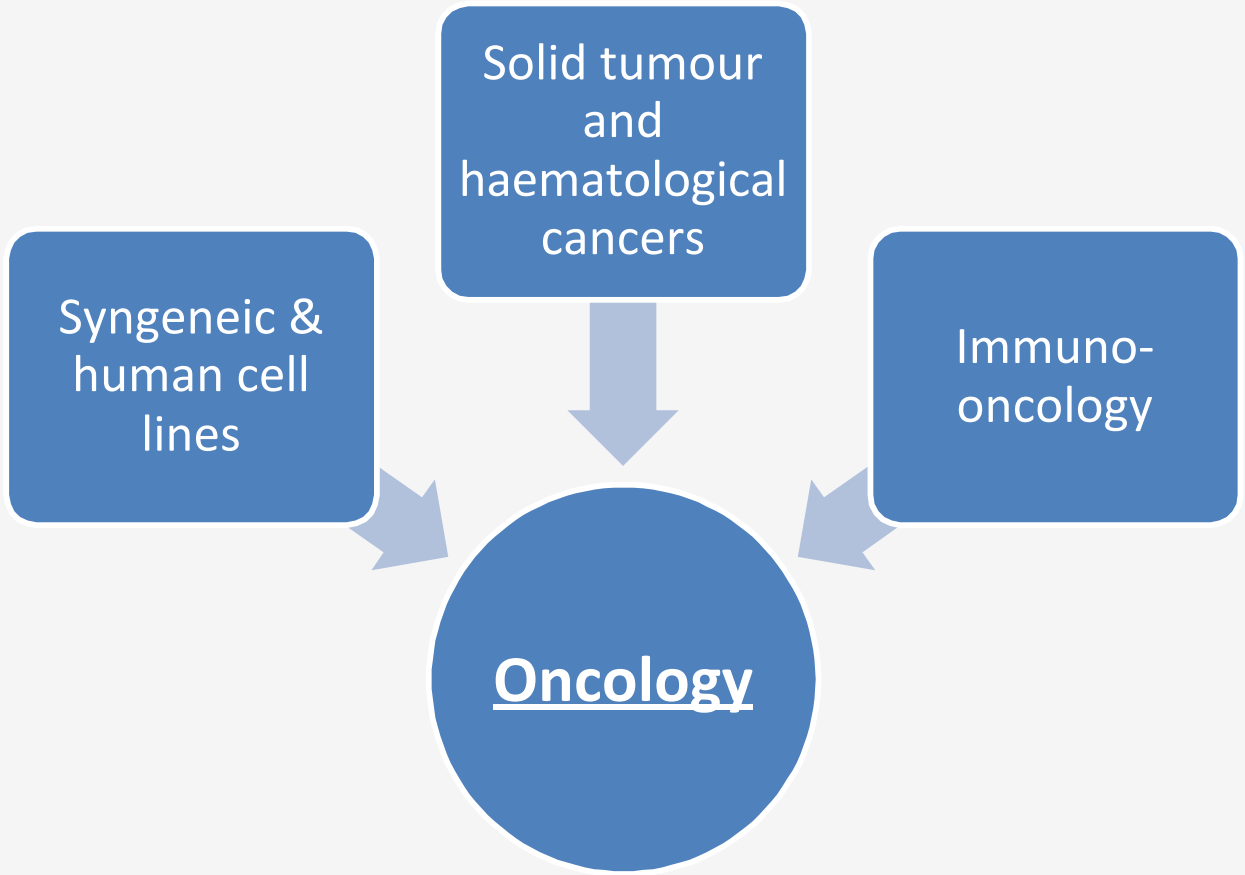
Oncology

- *In Vivo* Pharmacology
- Xenograft models
- Syngeneic models for Immuno-Oncology
- *In vitro* assays/cell biology

FACS

- Cell cycle analysis
- Cytokine analysis
- T-cell, B-cell tumour infiltration

Therapeutic Focus: foundation built around oncolo



>70 validated cell lines.

Cell biology through to in life an post in life analysis:

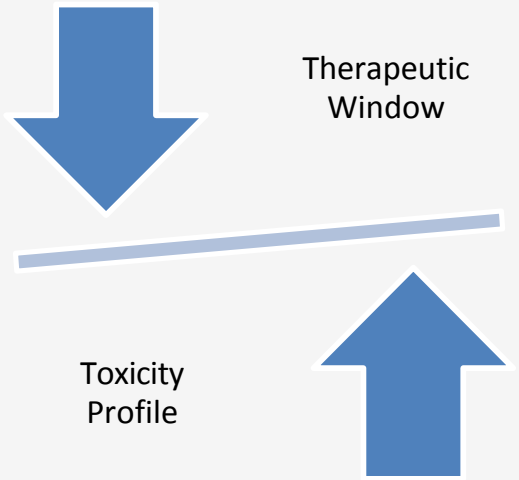
- Blood counts
- Blood biochemistry
- Histology
- PCR
- Western Blots
- ELISAs

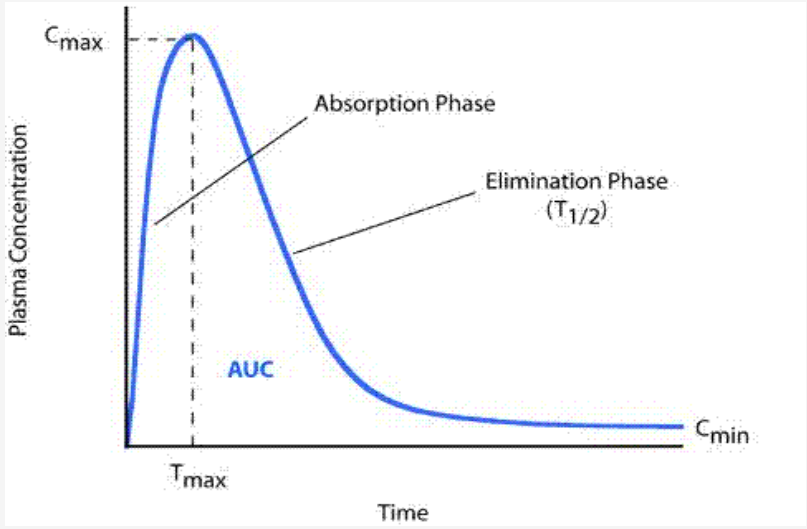
We deal with many different types of project, a rang clients, variations in approaches…..over the past 4 years IO demand has risen sharply!

- *Share observations: approaches that have worked well*
- *Key considerations*
- *Lots to consider!*

Aspects to Consider: Pharmacology

Prior to initiating IO studies, understanding the pharmacological profile of your compound is key!





In vitro data, IC50 determination, kinetics, dose levels, tolerability, dosing regimen…..

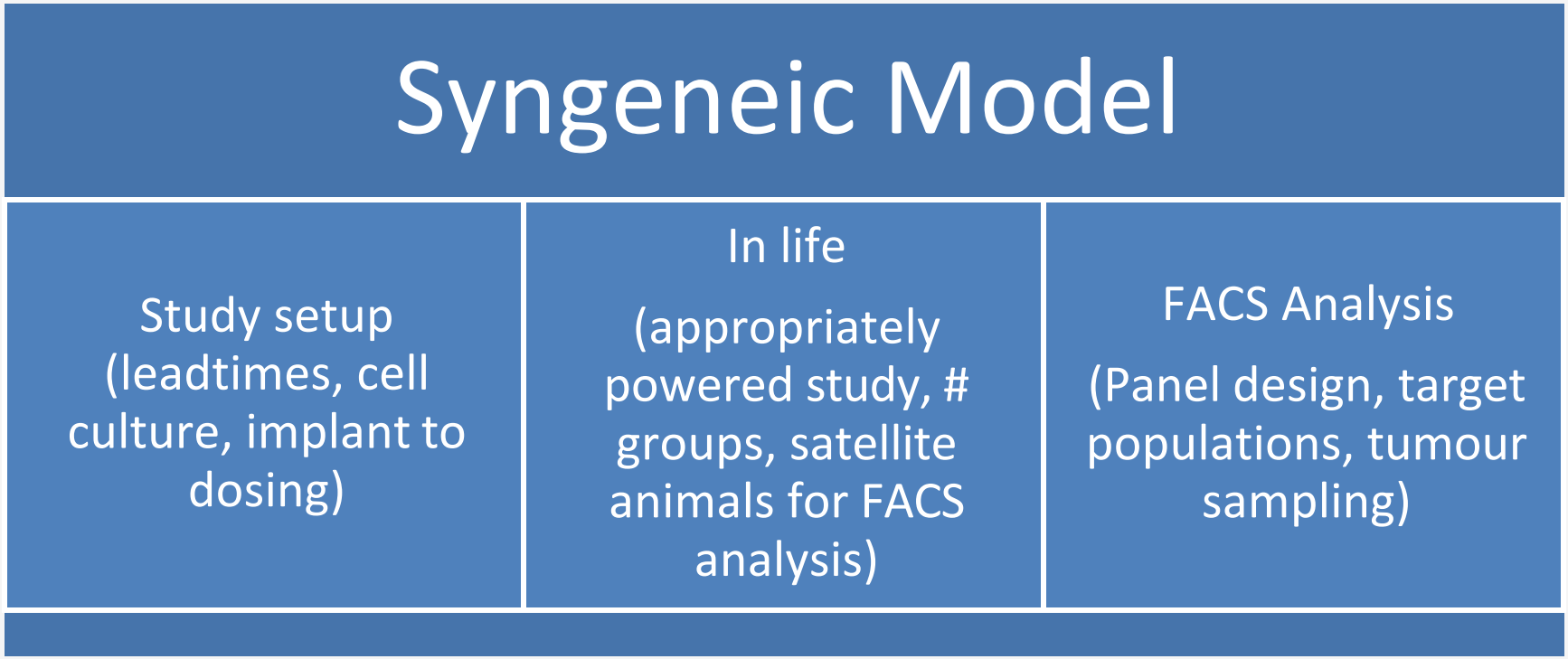
Immuno-oncology murine models

Model	Comments	
Syngeneic Models	Well established, quick to setup and run, relatively inexpensive vs. other models. Wild type in-bred strains e.g. C57BL/6, BALB/C	Functional aspects of murine human immune systems
Genetically Engineered Models	Specific mutations within the model to provide spontaneous tumour development – higher relevance to human tumours e.g. P53	High variability between stud animals, ease of setup and tir taken to data read out
Humanised Models	Engraftment of human cells into NOG mice e.g. PBMCs, HSCs	Longer term read out, relativ expensive vs other models, b clinically robust

- **Understand w hat questions you are trying to answ r: specifically the '*need knows*' vs the '*nice to haves.*'**
- **Select the most relevant model based on your individual compound, multipl factors at play (stage of development, budget etc).**
- **Speak to specialist CROs for input on approach and design.**

Syngeneic Murine Models

- **Timelines around cell culture understood (cell culture and implant → dosing)**
- **Limited variability vs other models.**
- **Quick models to establish and obtain data readouts.**



Syngeneic Murine Models

Syngeneic Model: learnings from in life setup

Successful Projects!

Understanding of pharmacology, kinetics and tolerability

Partnership approach with CRO: discuss existing data, get input and design on optimal approach

Select suitable and relevant model to your compound

Ensure the model is setup to provide the necessary endpoints

Analysis of target immune markers: build in satellites and ensure seamless through to FACS analysis

Analysis of Immune Markers

Tumour Microenvironment

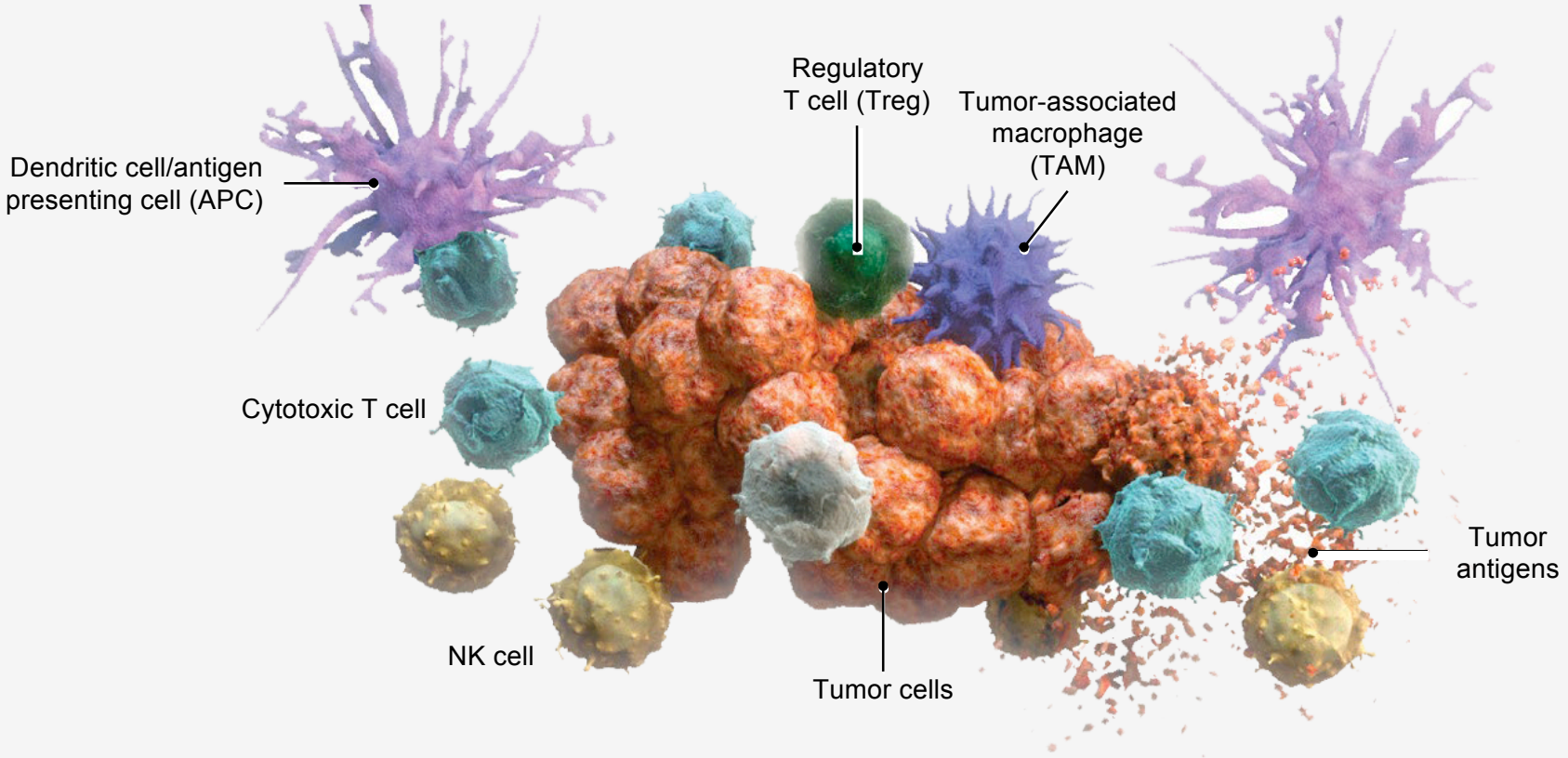


Image: Bristol-Myers Squibb

IMMUNE SYSTE

- Recognise, ta
  eliminate tun

  through the i
  adaptative sy

Targeting pathways that modulate an immune response

Two main pathways to consider:

1) Innate: e.g. CD137
2) Adaptive: e.g. CTLA-4, PD-1.

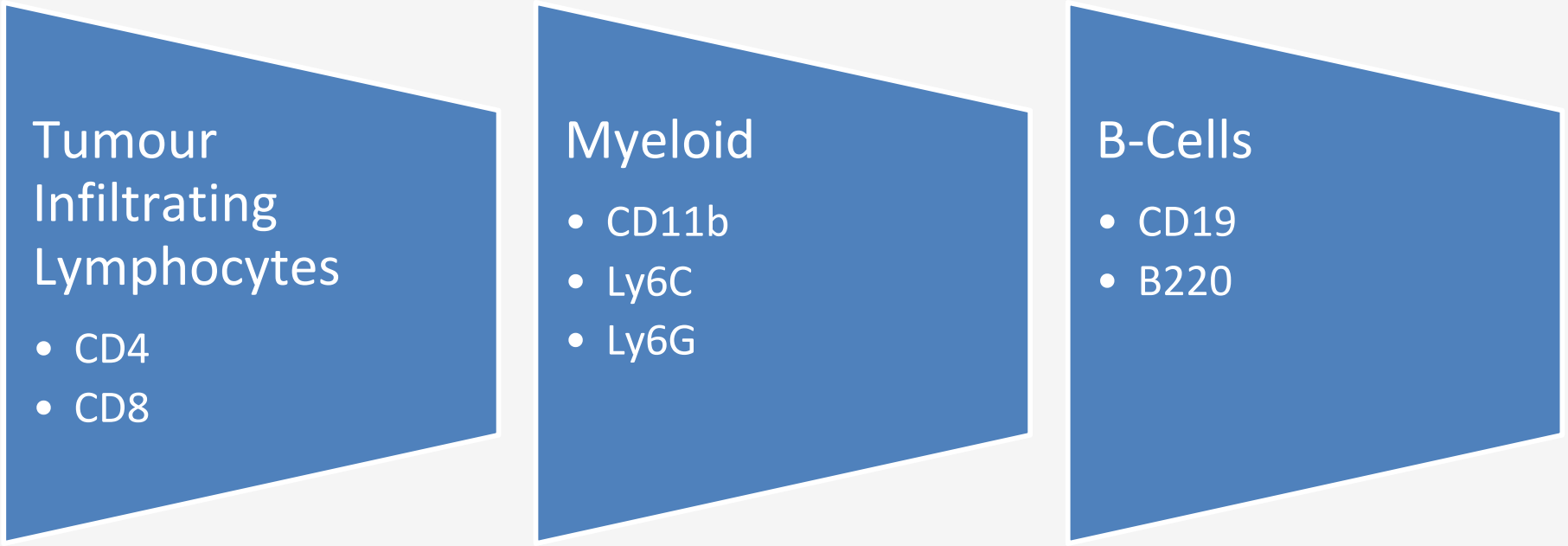
<u>Combination Targets:</u>

- ***Consider modulating two immune pathways to effectively activate the immune system i.e. vs a single modulatory pathway***
  <u>BUT</u>:

- ***Have a clear pharmacological rationale for use of combination treatments: e.g. direct and indirect pathways to provide anti-tumour effect.***

Immune Markers

Key markers of an immune response to consider:



- **The above are broad spectrum markers for the indicated populations.**
- **Other specific markers can be utilised i.e. more specific markers around apoptosis, cell exhaustion, cell activation etc.**
- **Ensuring flexibility in panel design is important.**

Assessing Impact on Immune Response: Analyses

Factors to consider for FACS:

Seamless from in life to analysis: sampling, validated protocols in place for each tissue of interest e.g:

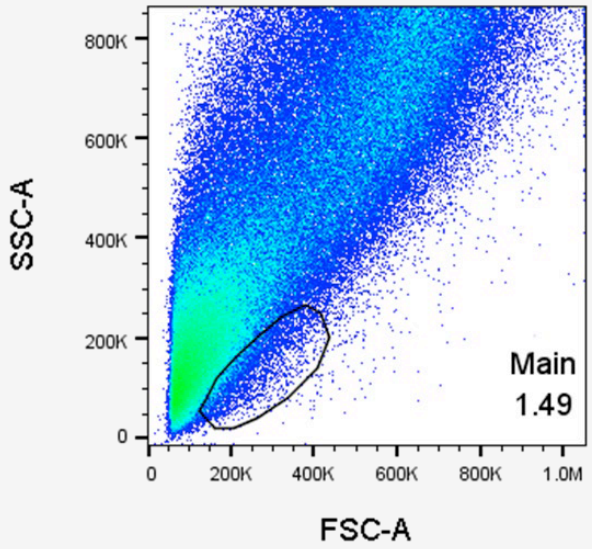
- **Specific tumour type**
- **Blood**
- **Tissue**

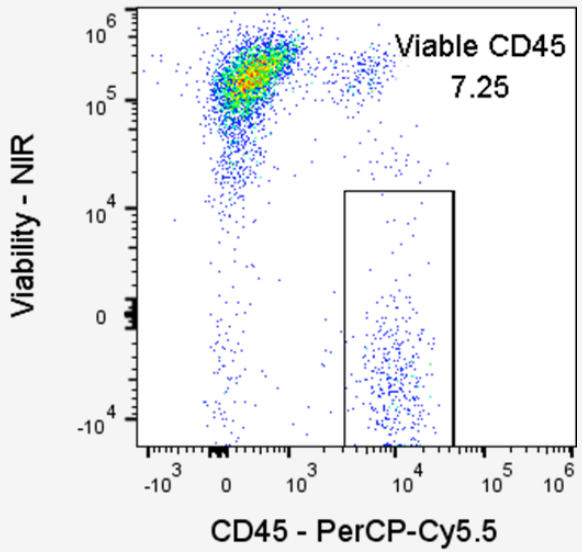
Complexities of different tumour types!
Satellite animals: lessons learnt around tumour volume.

Attune NxT Flow Cytometer (Thermo Fi

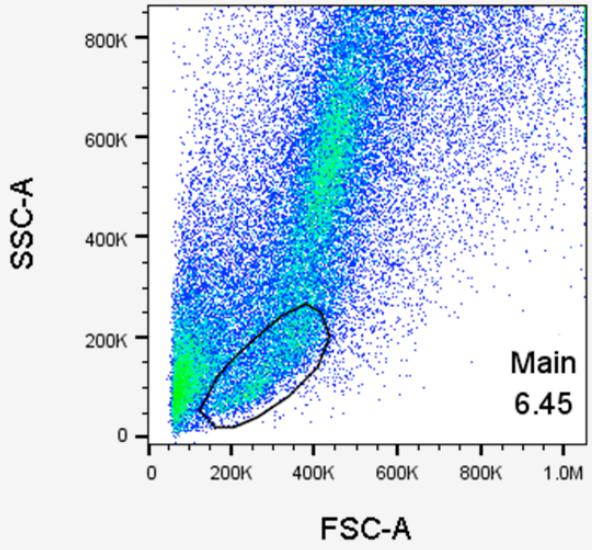
FACS considerations

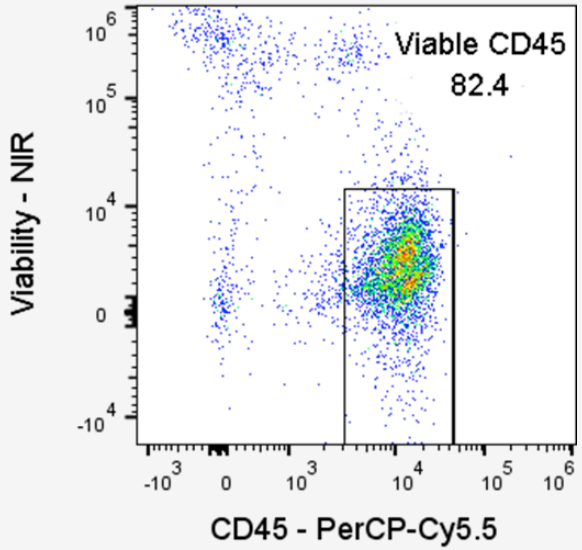
Without histopaque





With histopaque

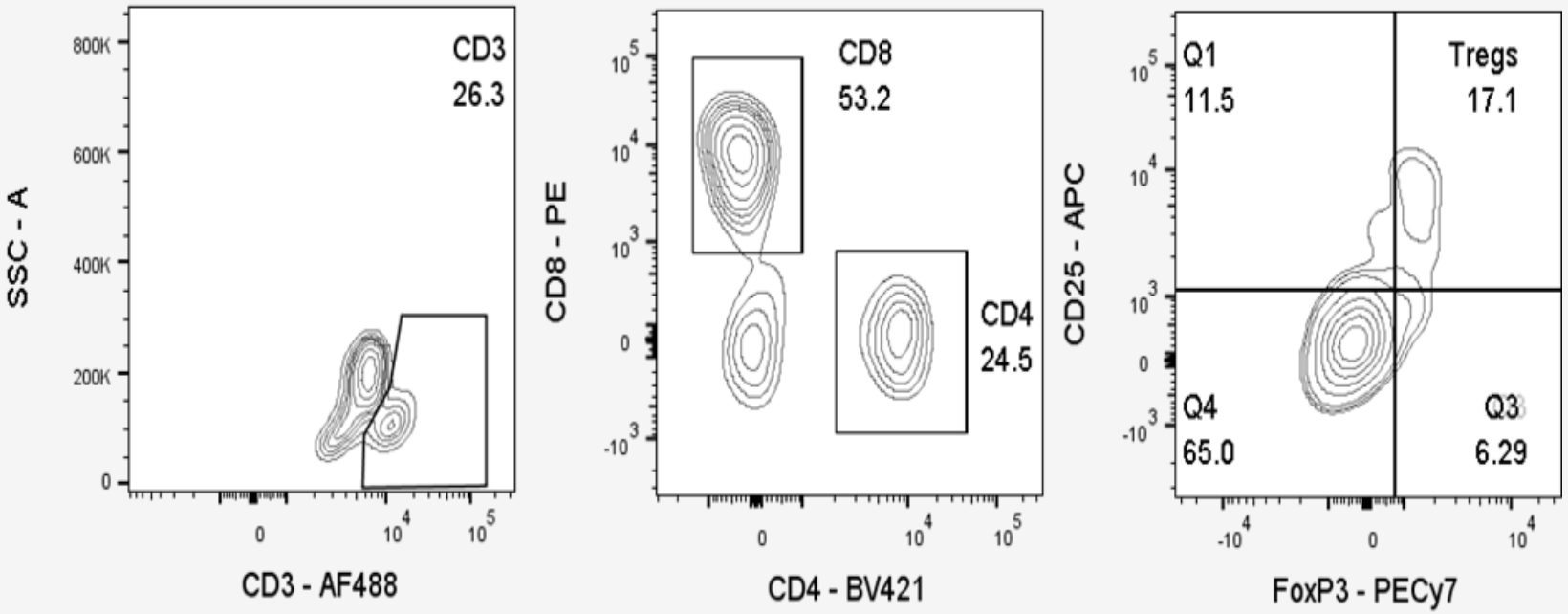




FACS considerations

With histopaque: number of events (lower number of events but increased targ
population of interest – less 'debris').

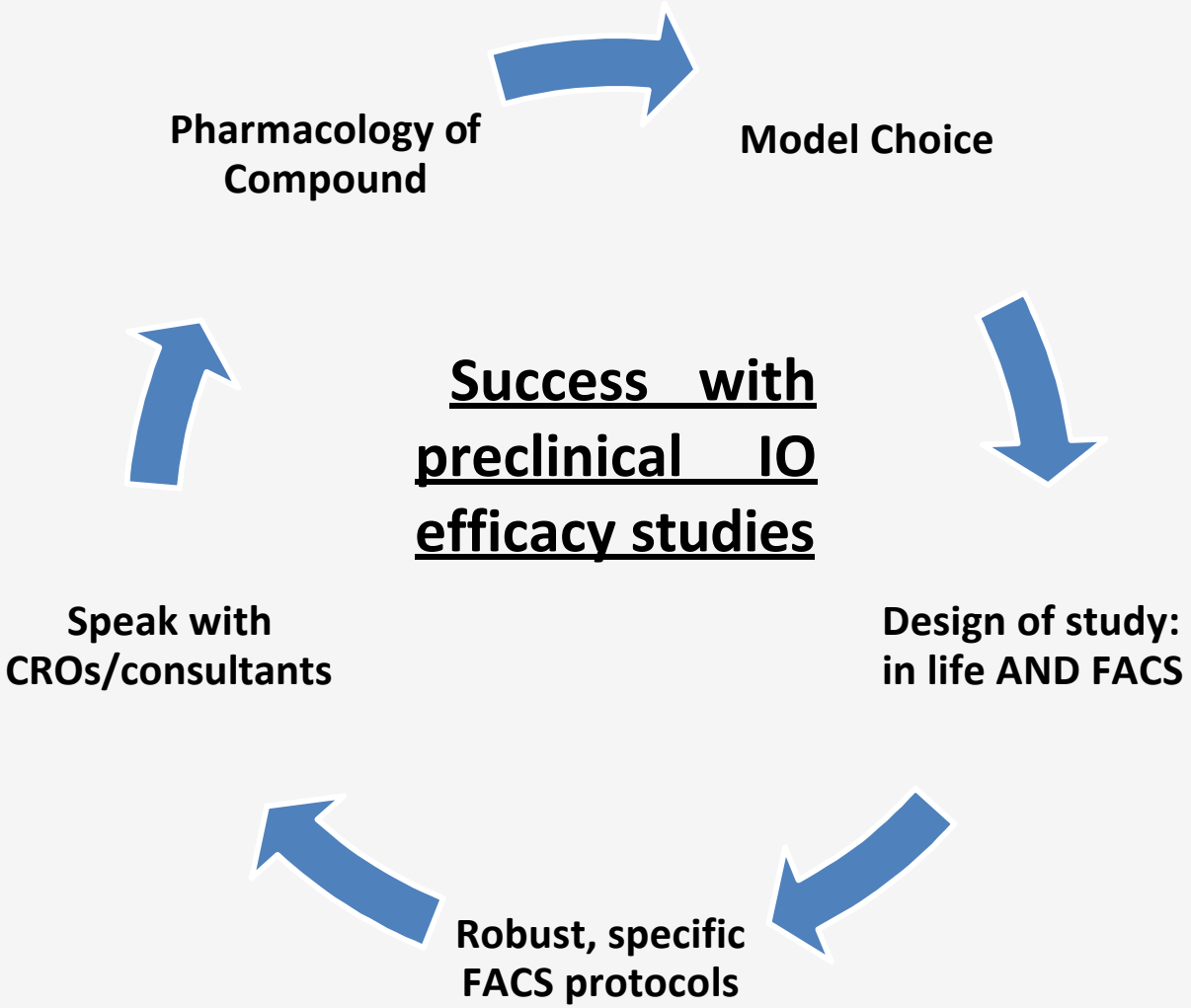
For all tumours e.g. T-cell analysis: T-reg population of interest (% of CD4 popul
Other markers around CD8 population e.g. IFNγ, PD1 etc.



Case Study


Existing Data review
FACS Analysis
IO
In vitro & in vivo pharmacology
Syngeneic Models

Concluding thoughts/take home messages



Final Slide

Email: ***johnny@axisbio.co.uk***

Final note: come and visit us in Northern Ireland, an amazing part of the world with lots to do and see!