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Development of an *In Vitro* Human Cell-based Assay to Investigate the Role of Lipids, Dendritic Cells, and Invariant NKT cells in Allergic Sensitisation

Georgie Hopkins



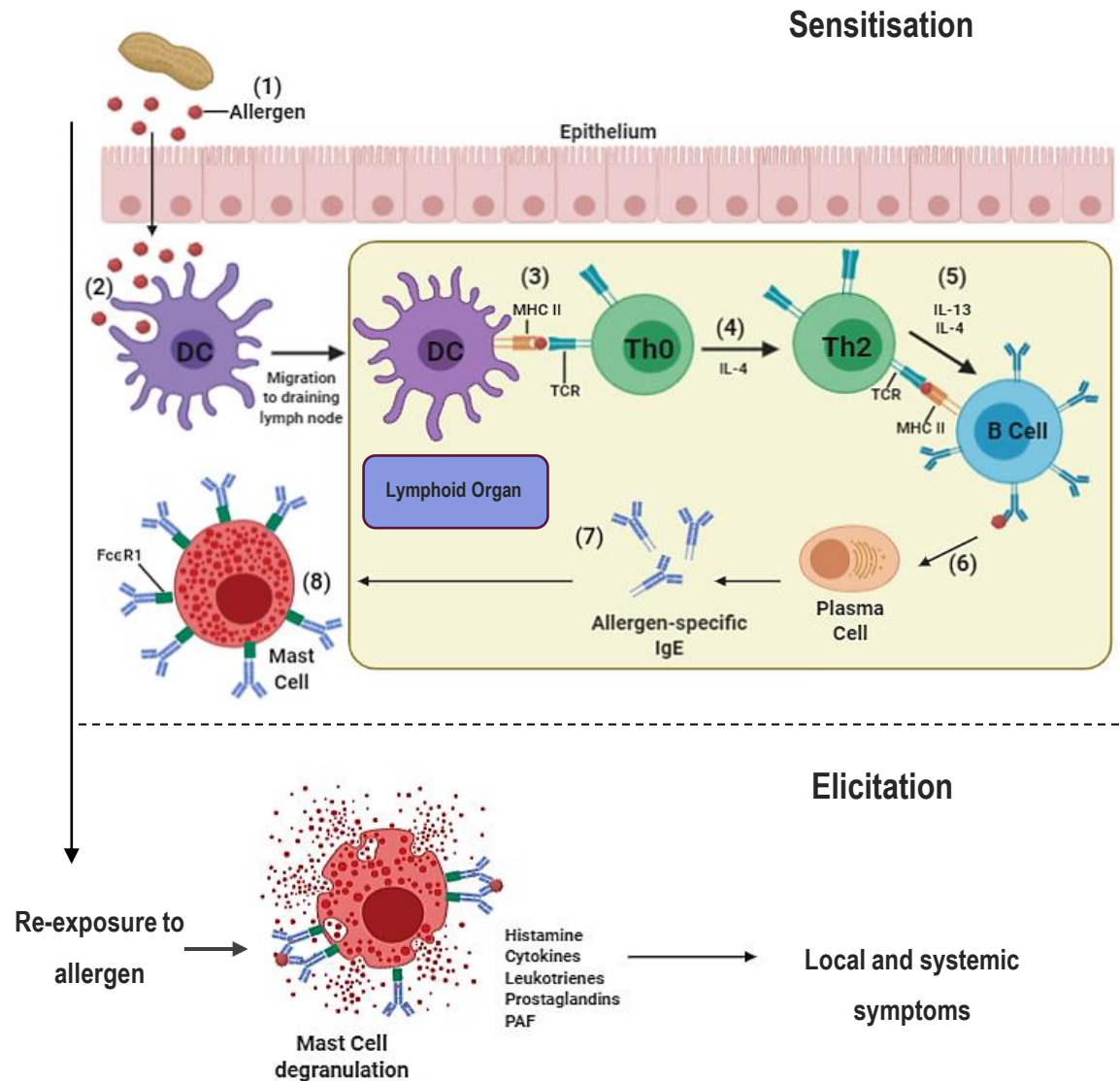
What is an Allergy?

- An allergy is an unnecessary immune responses to a harmless substance e.g. peanuts.
- Clinical manifestations include oedema, hives, itching, and in extreme cases, a systemic reaction called anaphylaxis shock, which can be fatal.
- IgE-mediated allergies are increasing in prevalence, with IgE-mediated food allergies affecting up to 10% of children and 6% of adults worldwide.



Image taken from: allergy.uk/org

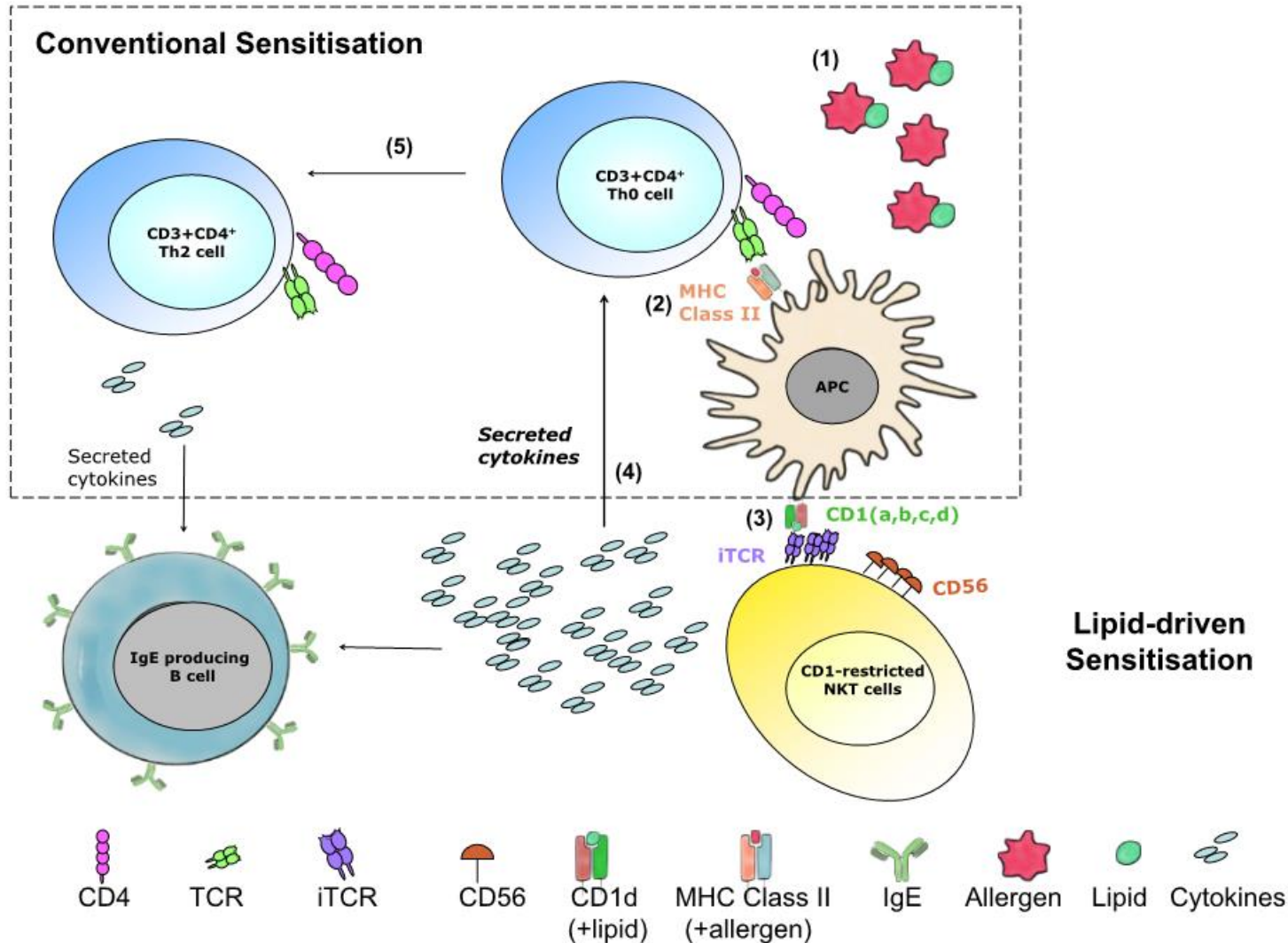
Allergic Sensitisation



- There are two phases to IgE-mediated allergy: **allergic sensitisation** and **elicitation** of symptoms.
- The first exposure to an allergen (sensitisation) causes the production of allergen-specific IgE, which bind to mast cells.
- Further exposure to the allergen (elicitation) results in the allergen cross-linking the existing bound IgE on mast cells, triggering mast cell degranulation and subsequent onset of symptoms.
- However, the mechanisms underpinning the first phase of IgE-mediated allergy, **allergic sensitisation**, are unclear.



Role of Lipids in Allergic Sensitisation



- Lipids are presented on CD1 molecules on dendritic cells (DCs).
- CD1-restricted natural killer T (NKT) cells recognise the lipid via a semi-invariant T cell receptor (TCR).
- These activated invariant NKT (iNKT) cells can release Th1 or Th2 cytokines.
- Recent evidence suggests lipids can induce the release of Th2 cytokines to result in a Th2 response.



- 1. To investigate the role of lipids in the development of allergic sensitisation, utilising a human model**
 - To optimise all techniques required for the eventual measurement of Th1 and Th2 cytokines from invariant NKT cells.
 - The lipid, α -GalCer, will be used in developing this assay as it is the most potent iNKT cell activator.

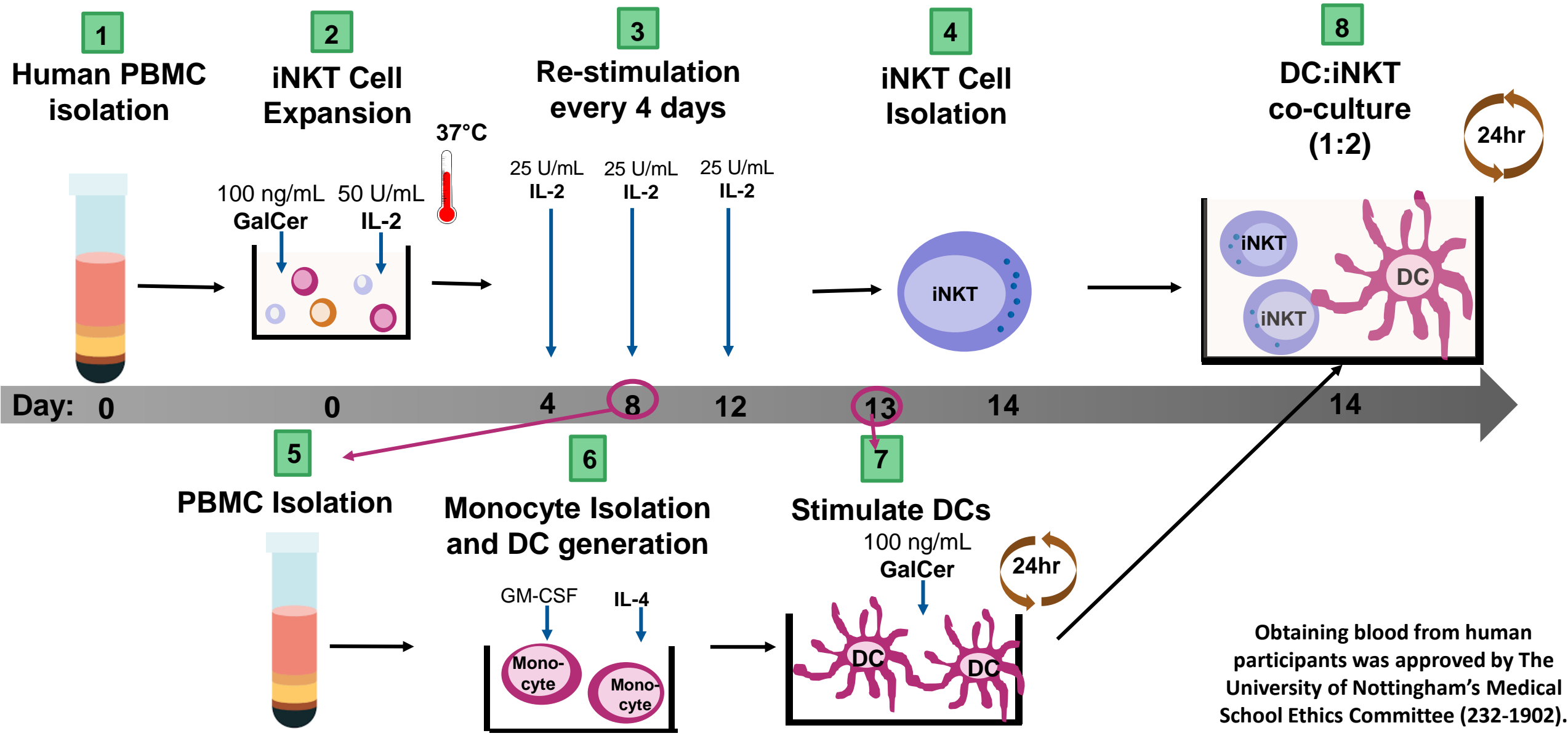


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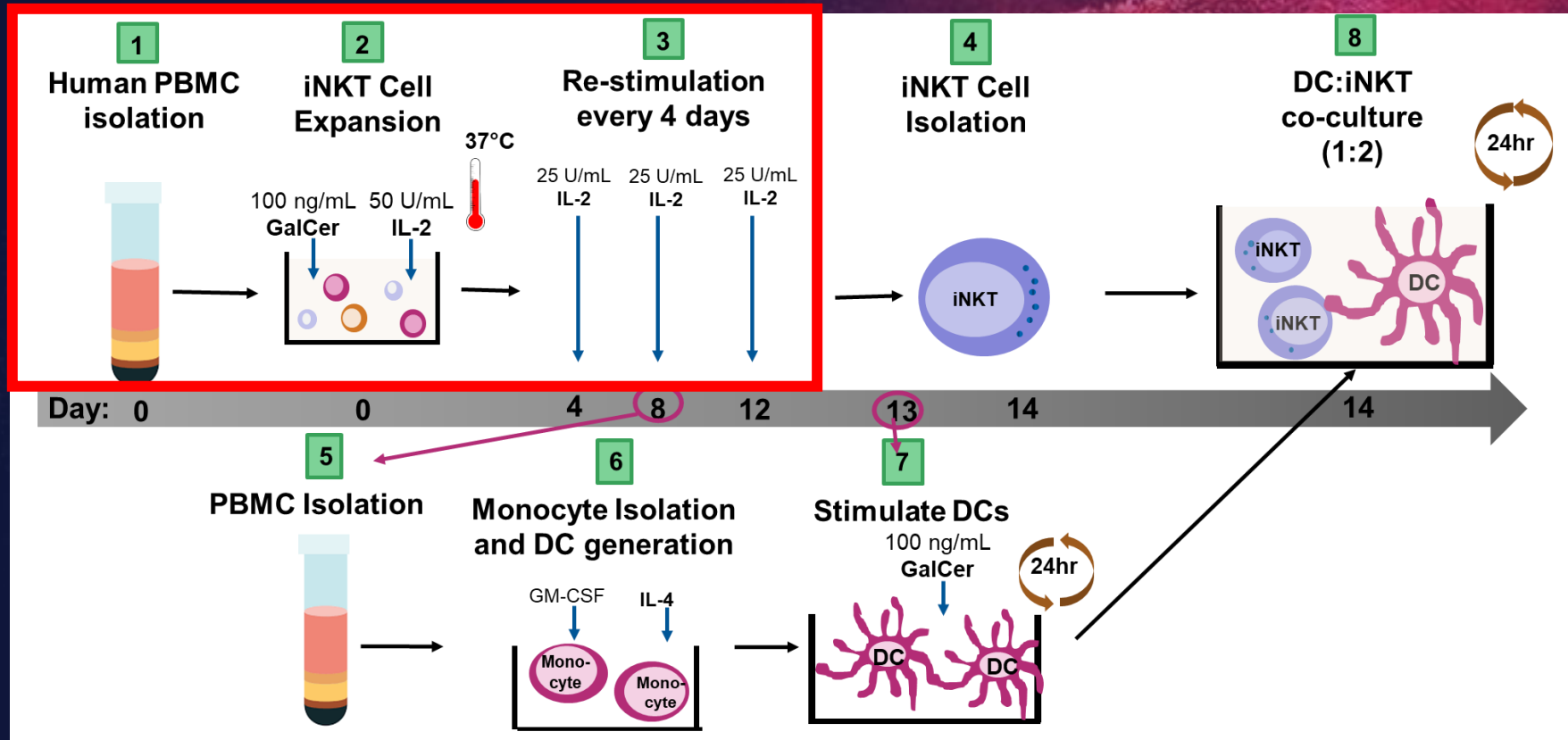
Methods



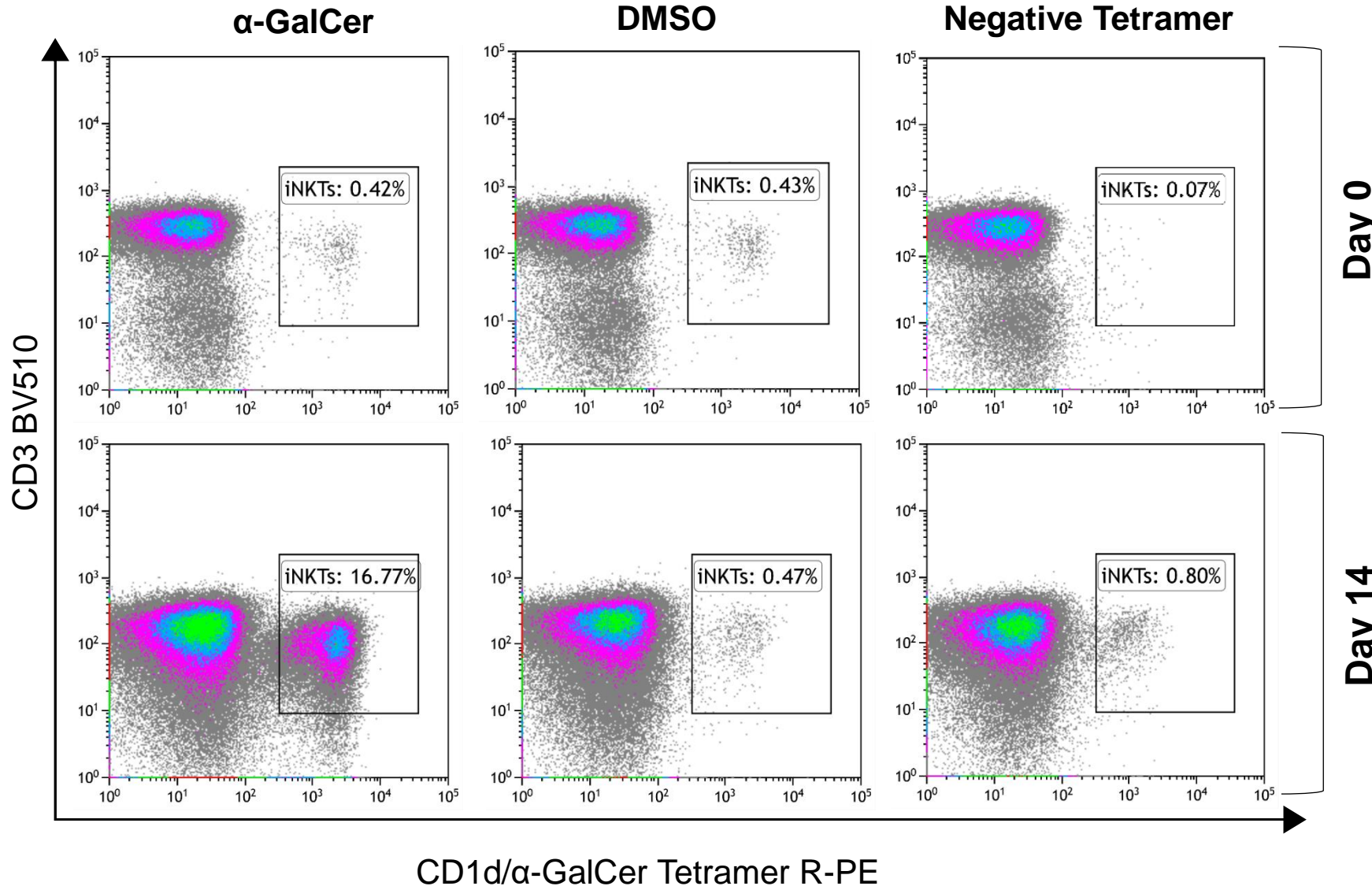
Obtaining blood from human participants was approved by The University of Nottingham's Medical School Ethics Committee (232-1902).

Results

iNKT Cell Expansion



iNKT Cell Expansion

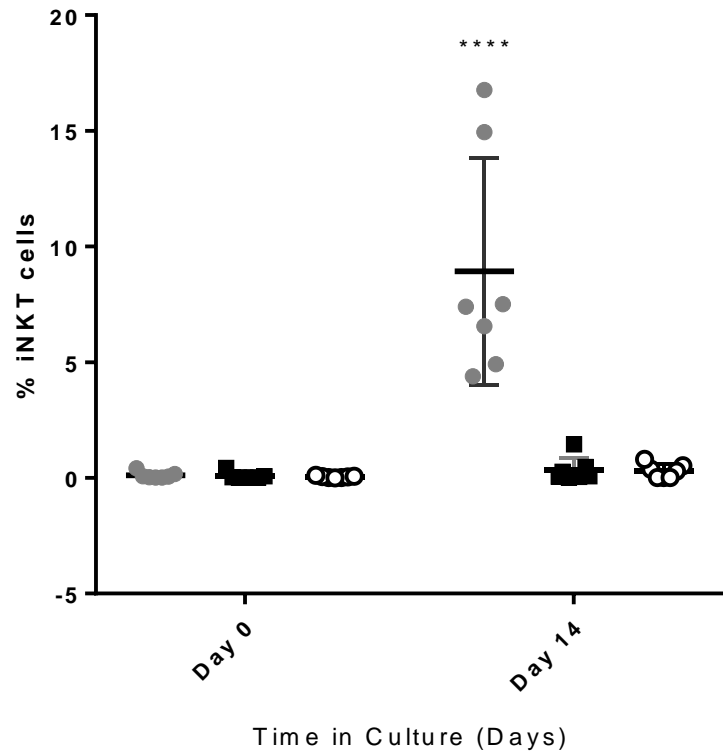
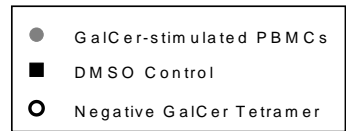


GalCer stimulated iNKT expansion from **0.42%** on Day 0 of culture, to **16.77%** of CD19 negative lymphocytes at Day 14.

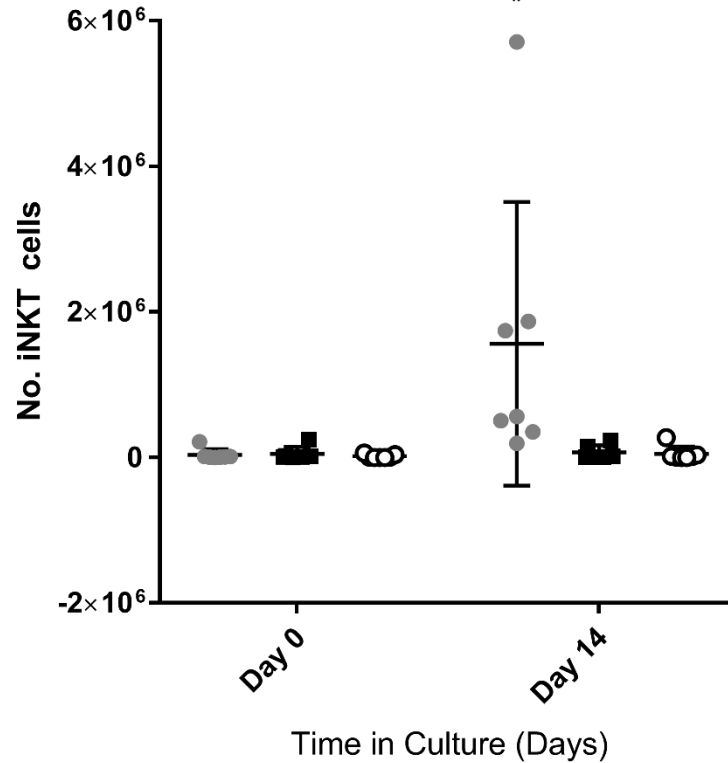
The DMSO control did not induce iNKT expansion.

Staining with the blank-loaded CD1d tetramer showed negligible false-positive binding of the tetramer.

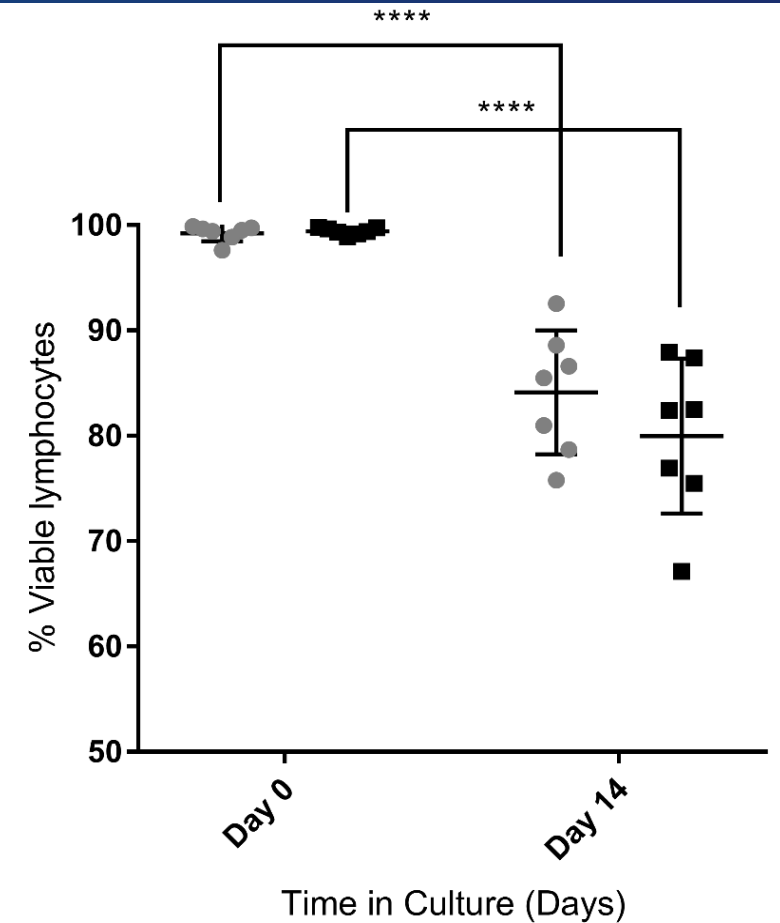
iNKT Cell Expansion



(A) α -GalCer induced a mean proliferation of **0.11%** iNKTs on Day 0, to **8.92%** on Day 14.



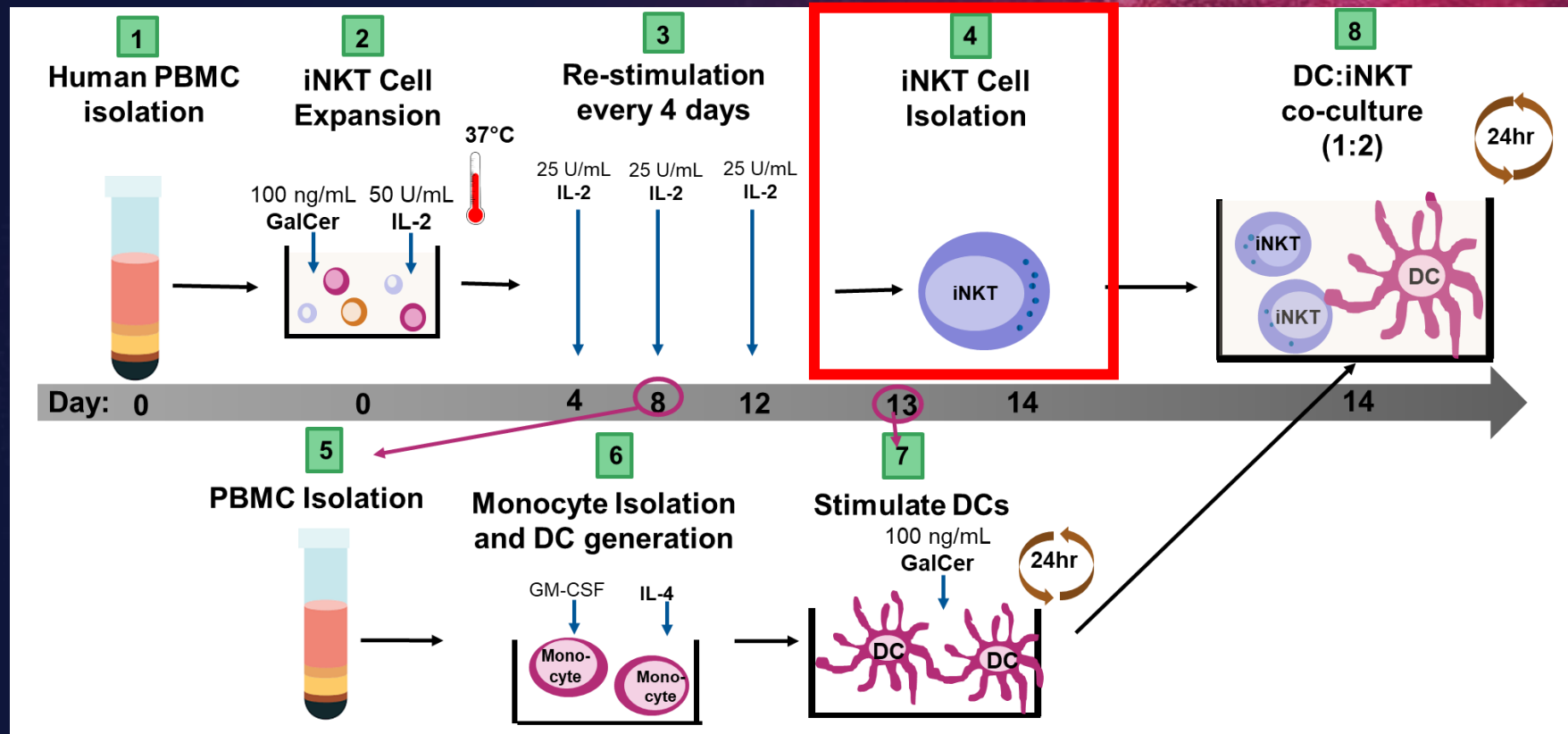
(B) **37,143** iNKT cells on Day 0 increased to **1,562,598** by Day 14 of culture with α -GalCer.



(C) The viability of lymphocytes began at **99.25%** and decreased to **84.12%** for α -GalCer-stimulated PBMCs at Day 14, and **79.98%** for the DMSO control.

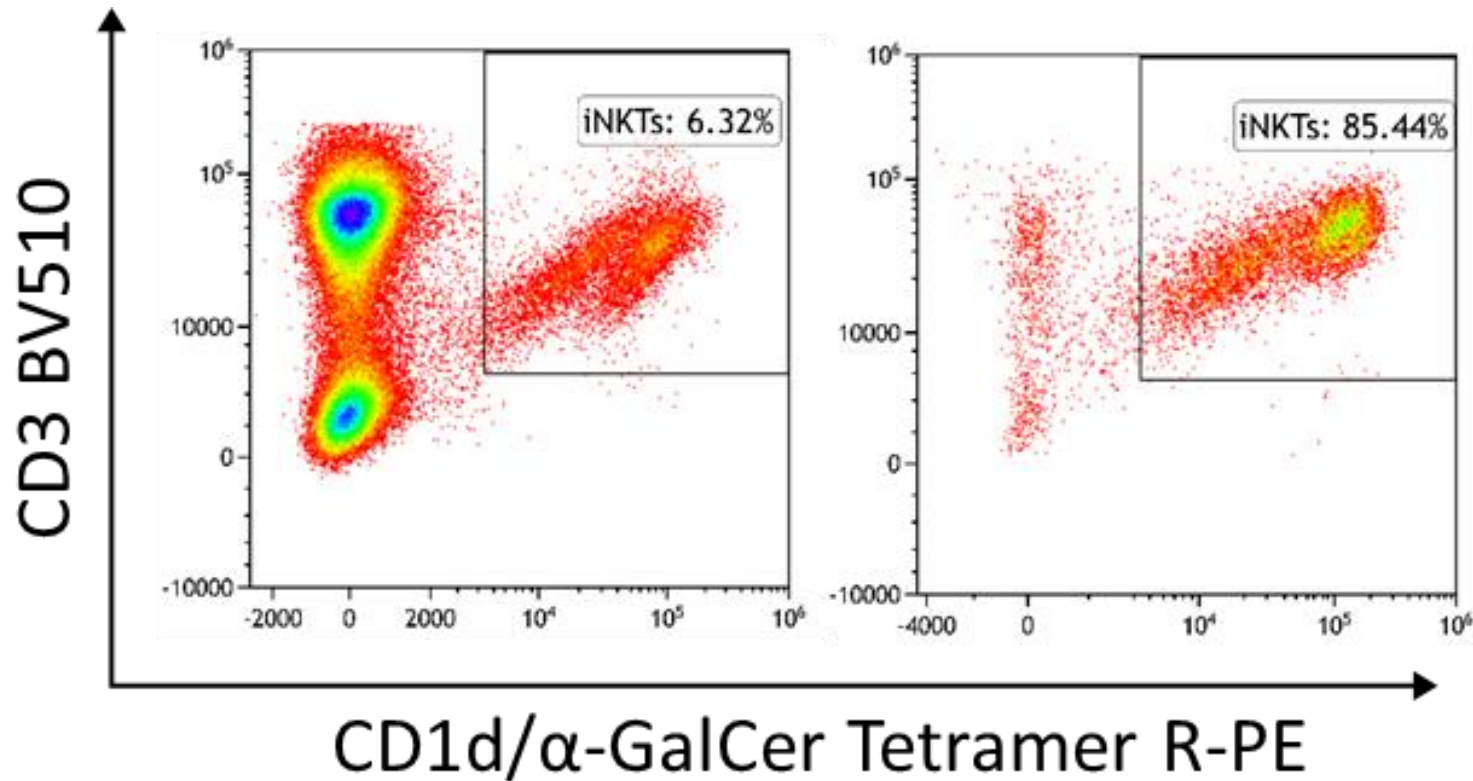
Results

iNKT Cell Isolation





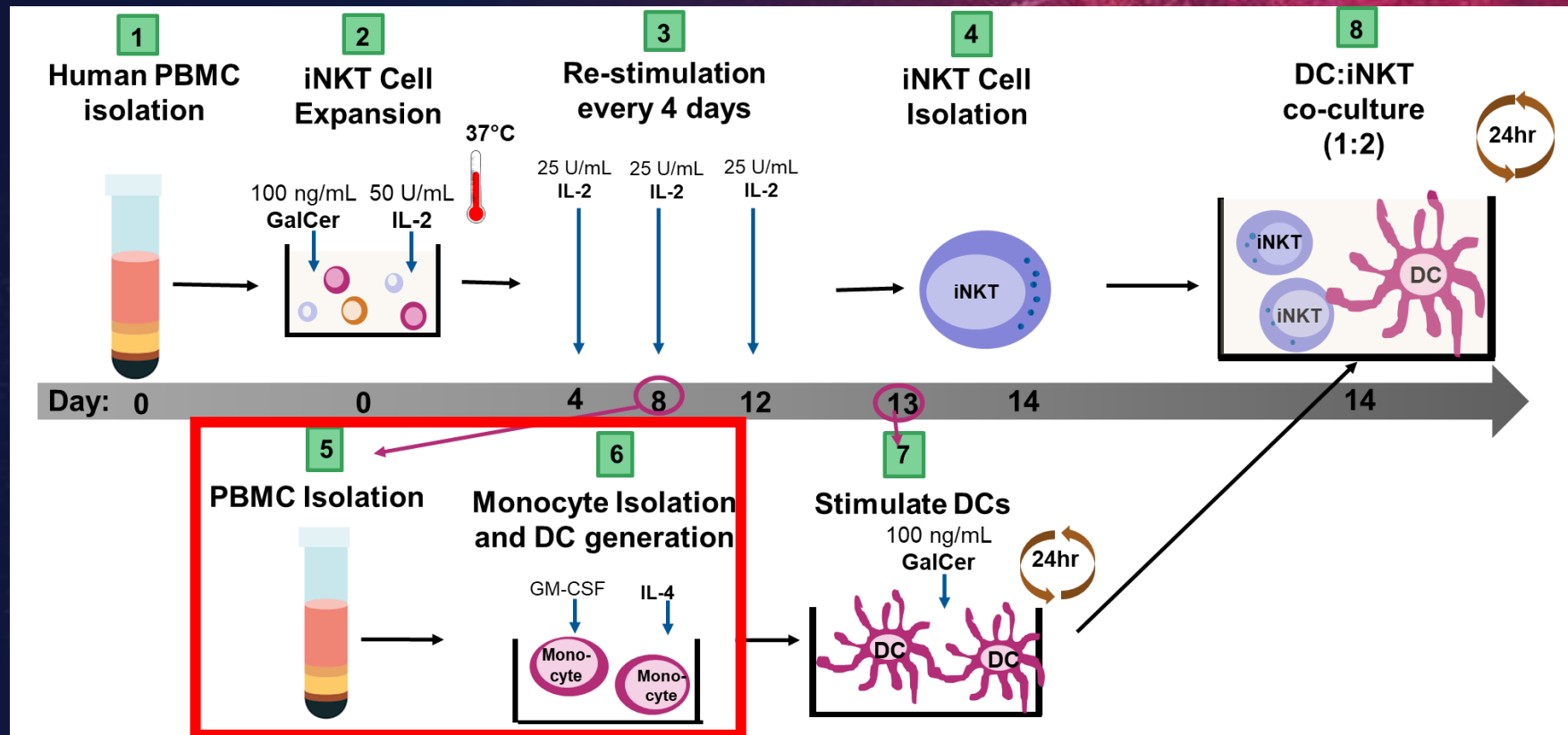
iNKT Cell isolation



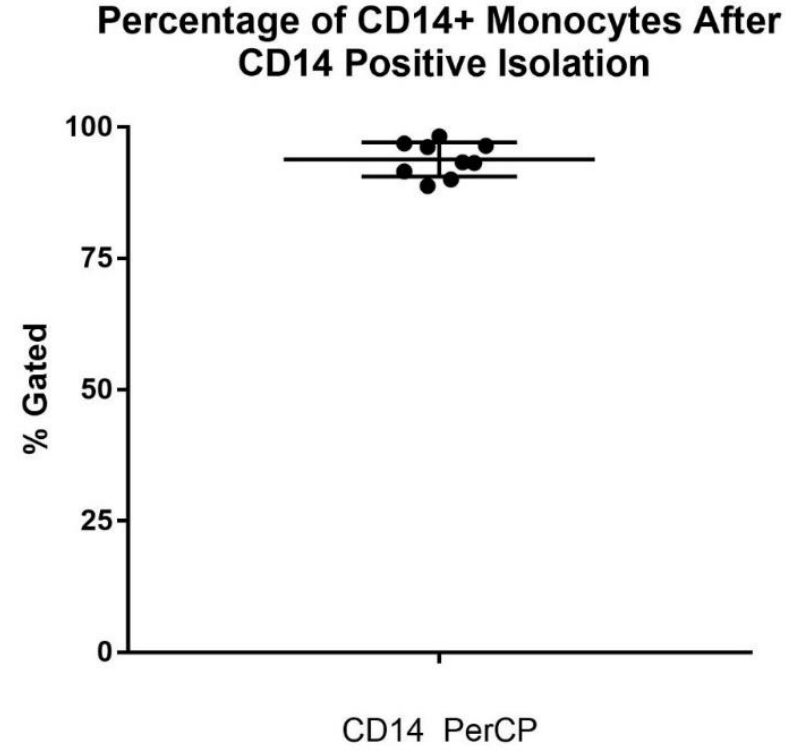
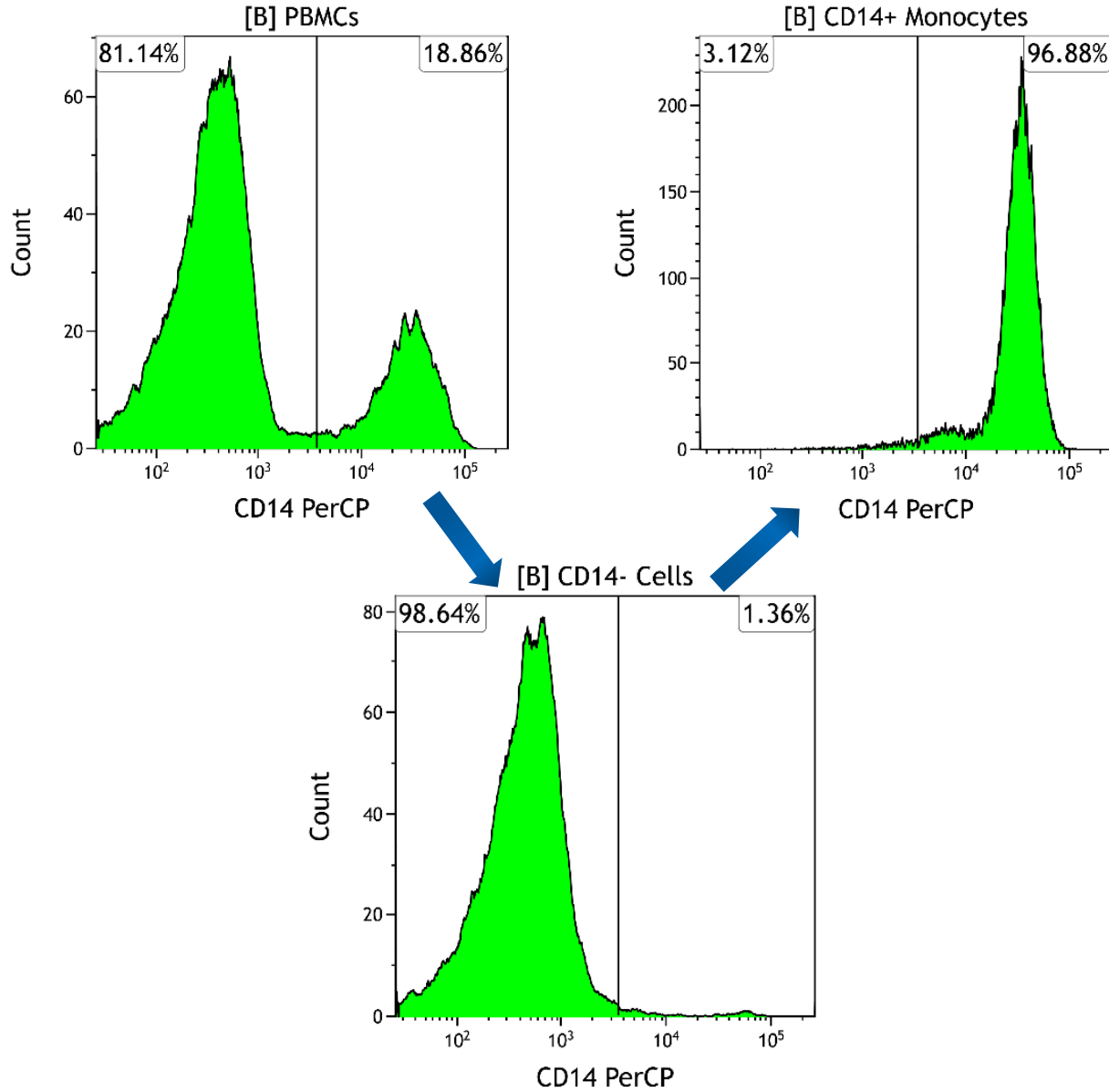
On Day 14 of iNKT expansion, iNKT cells were isolated using anti-PE microbeads and immunomagnetic isolation, resulting in a purity of 85.44%.

Results

Monocyte Isolation + DC Generation



CD14+ Monocyte Isolation

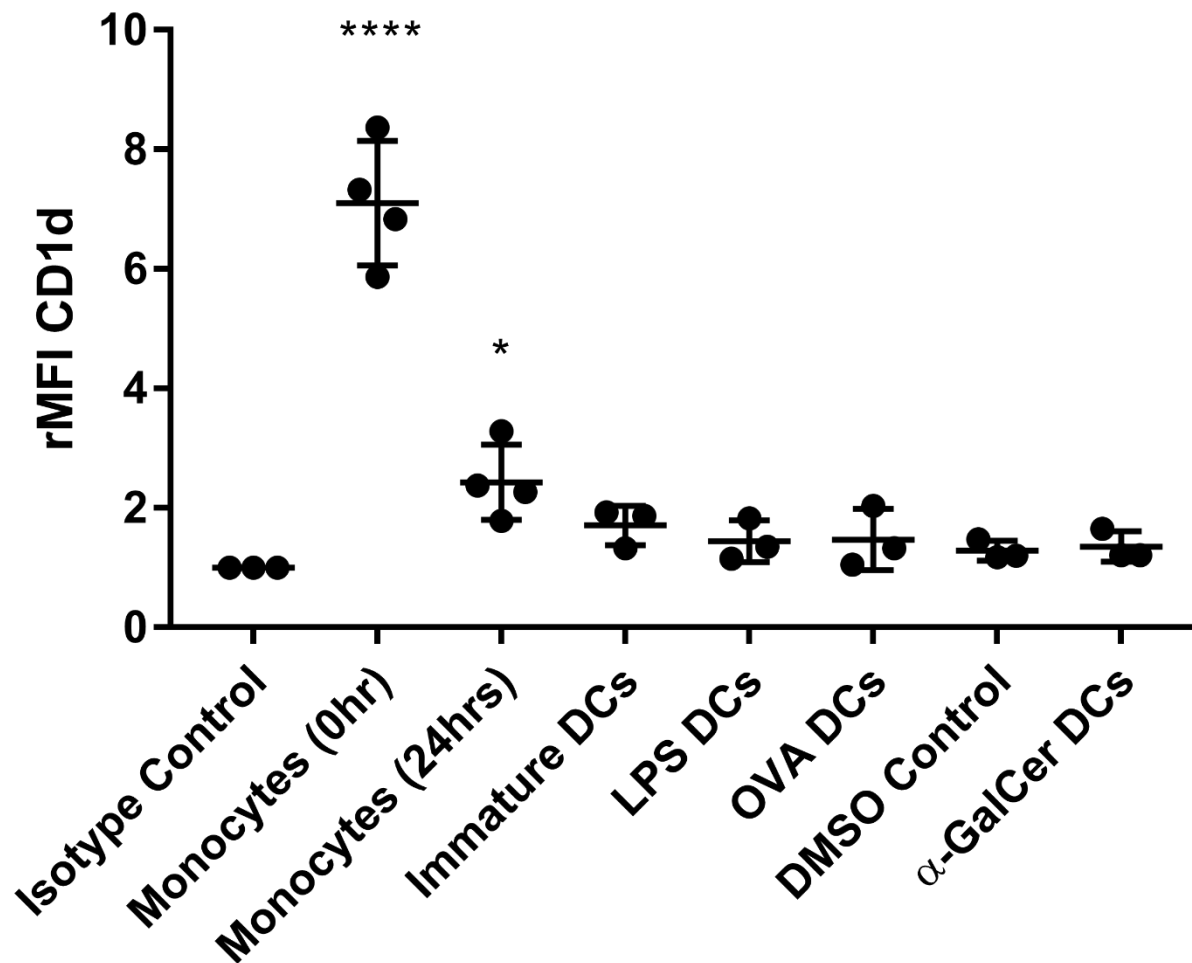


- PBMCs were isolated and incubated with CD14 Microbeads. The cells were then immunomagnetically isolated using a MACS column.
- Average of **93.86%** (n=9, SD=3.30) purity after CD14⁺ isolation.



CD1d Expression

- CD1d expression was measured as it is essential to present lipids to iNKT cells.
- The data showed CD1d expression is significantly downregulated after monocytes are placed in culture.
- Is it a result of media components?



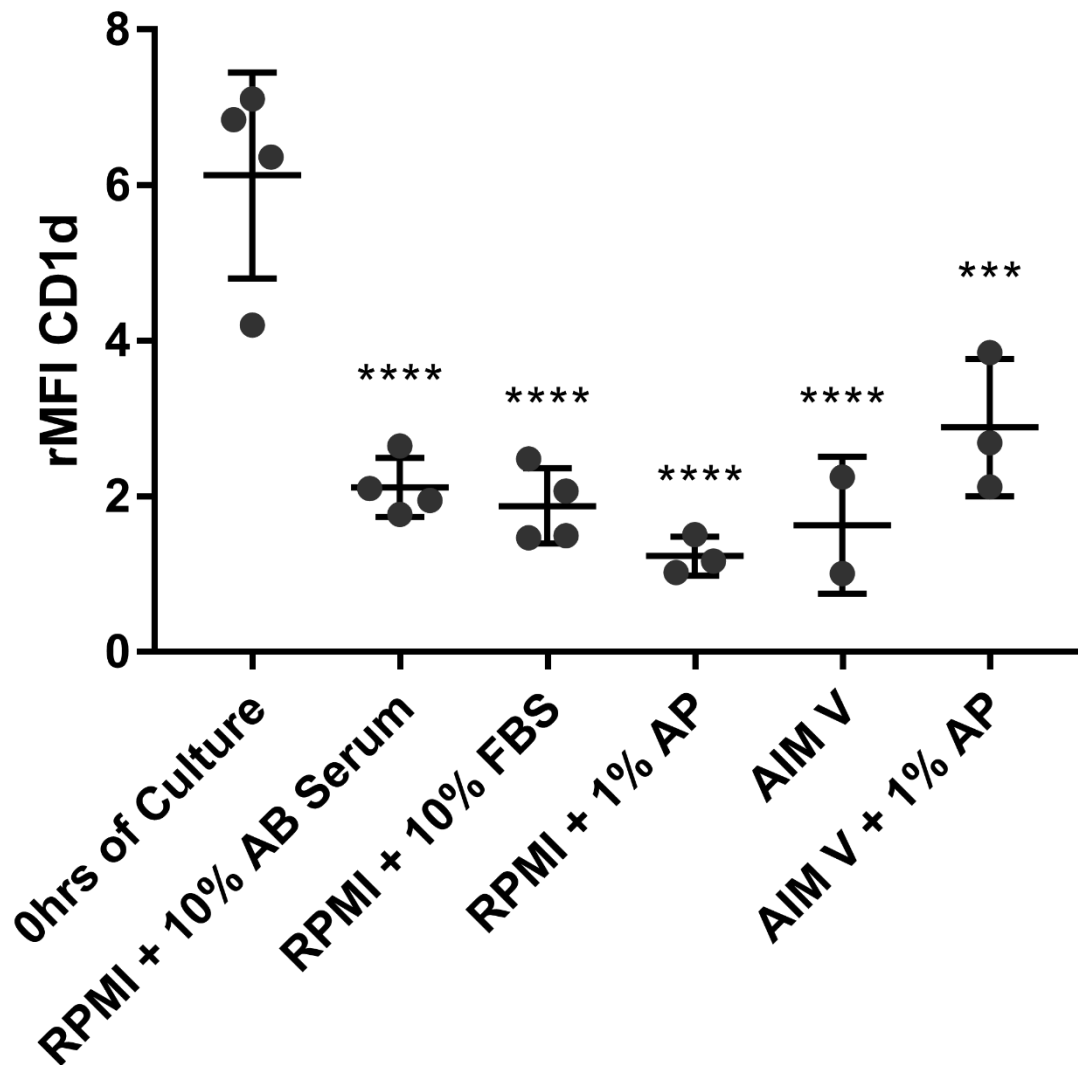


CD1d Expression in different medias

CD1d Expression was still significantly downregulated in all different medias.

But it was least downregulated when cultured in **AIM V + 1% autologous plasma**, followed by **RPMI + 10% human AB serum**.

Viability data showed **RPMI + 10% AB** was optimal.



Results

Stimulate DCs with Lipid

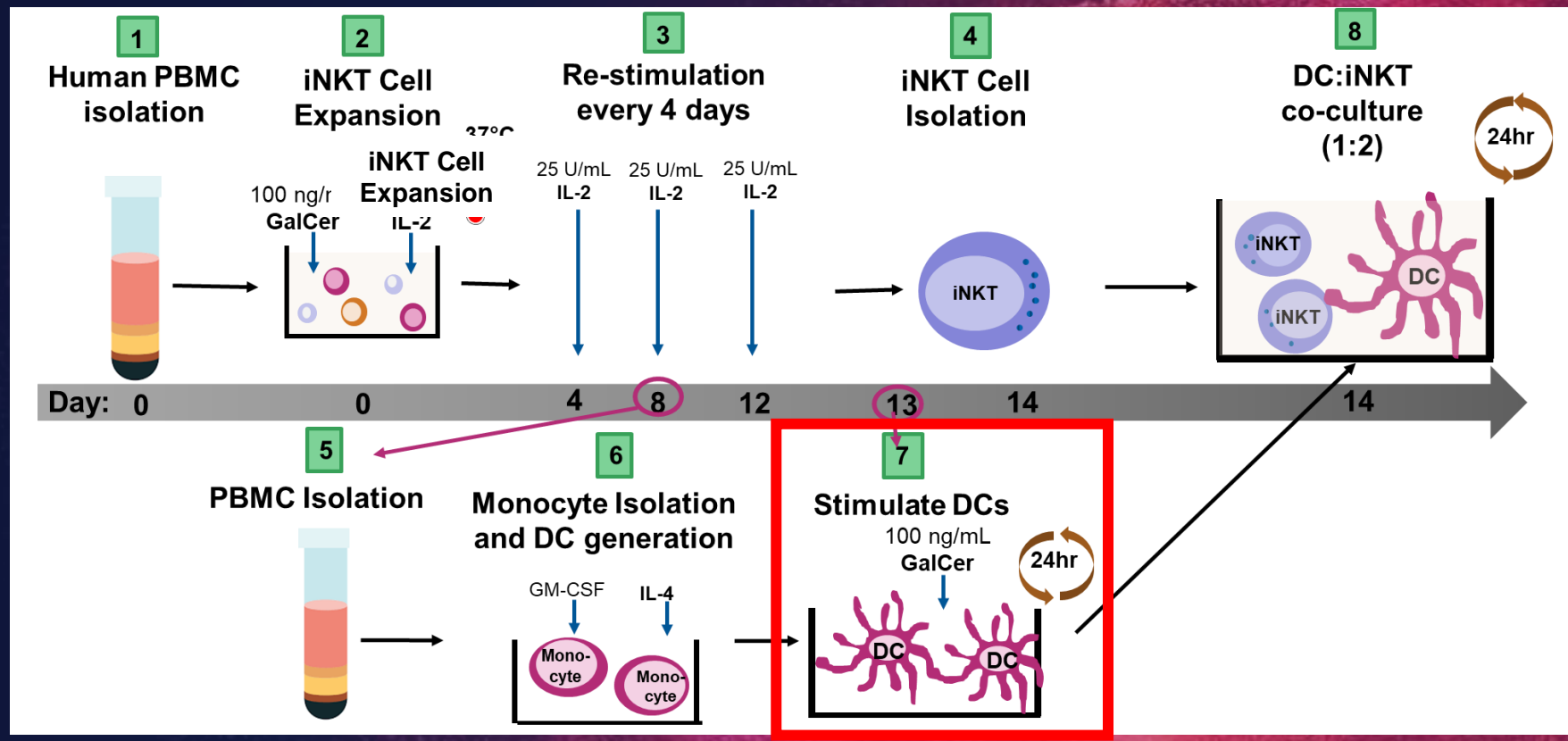
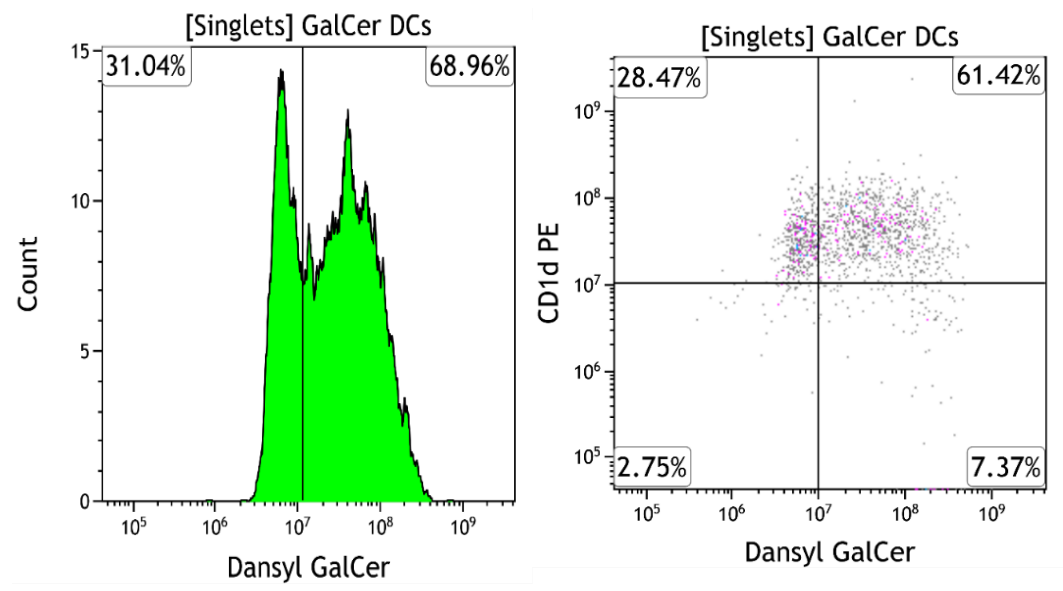
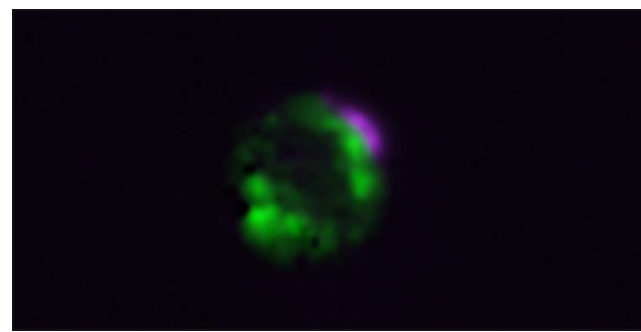


Image Stream DCs

A.

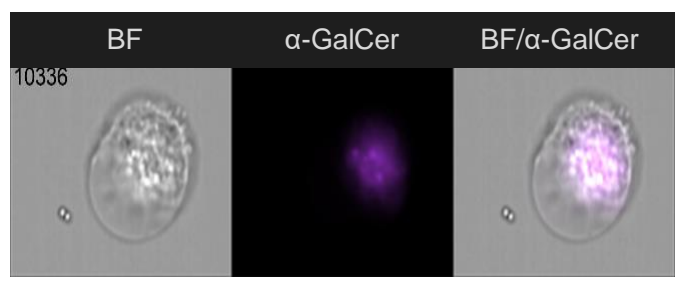


B.

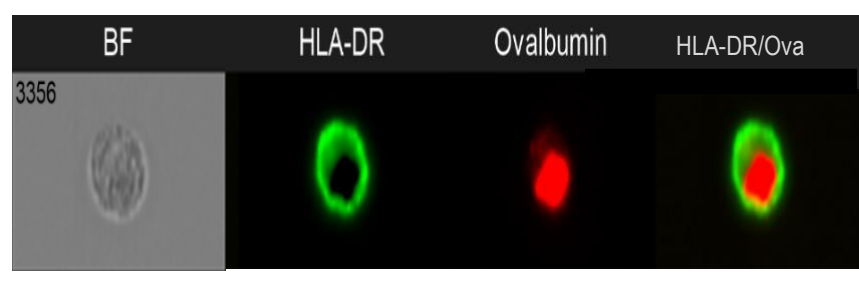


- Fluorescent α -GalCer was internalised by immature DCs (iDCs) and co-located at lysosomal compartments.
- The protein, Ovalbumin, was also internalised and associated with MHC II.

C.



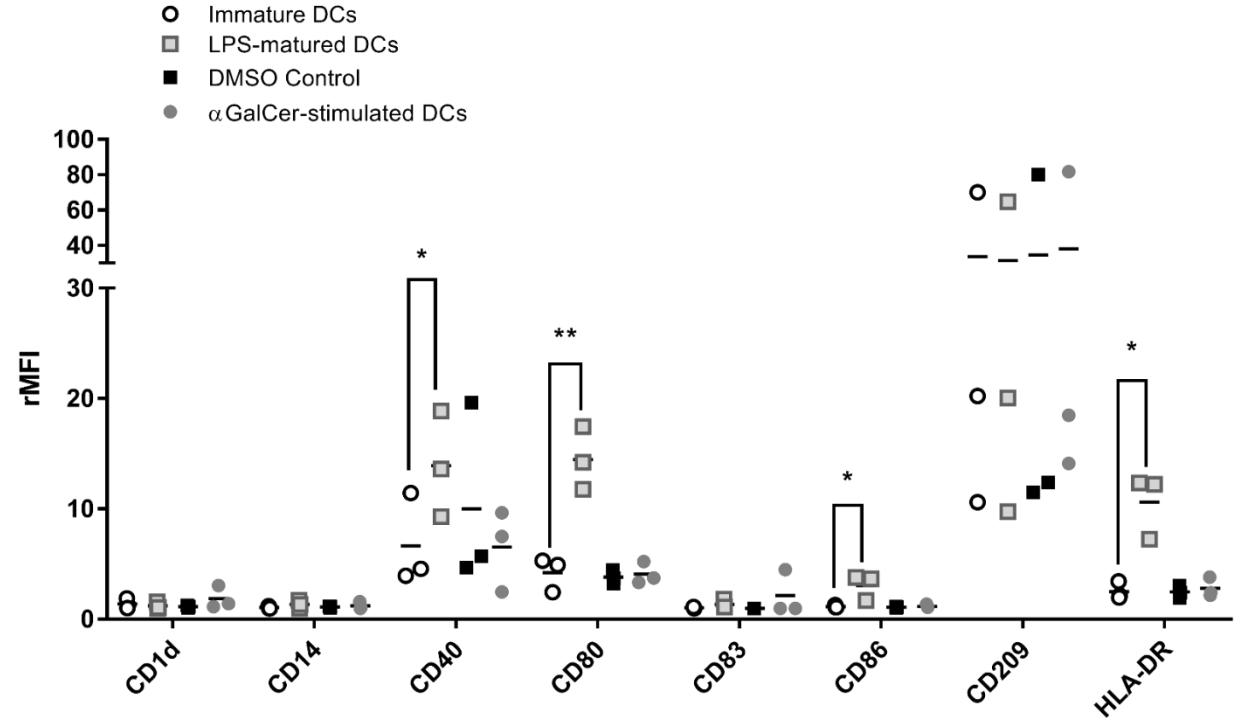
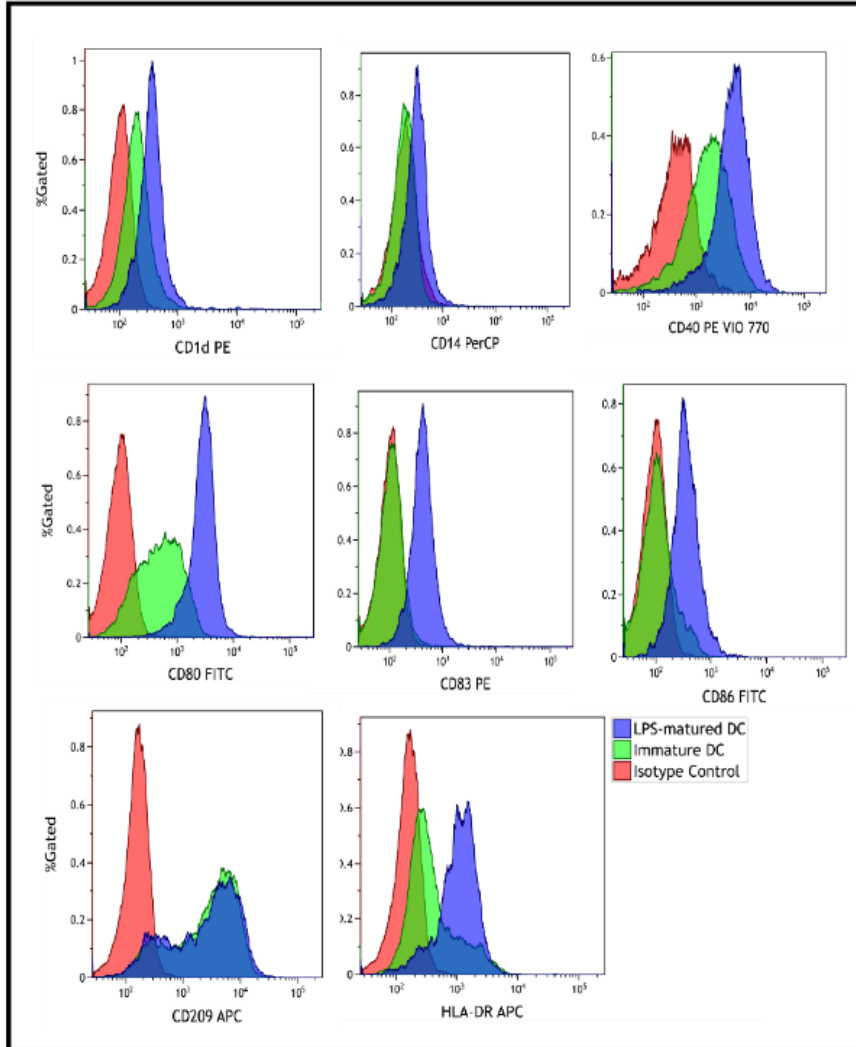
D.





DC Generation

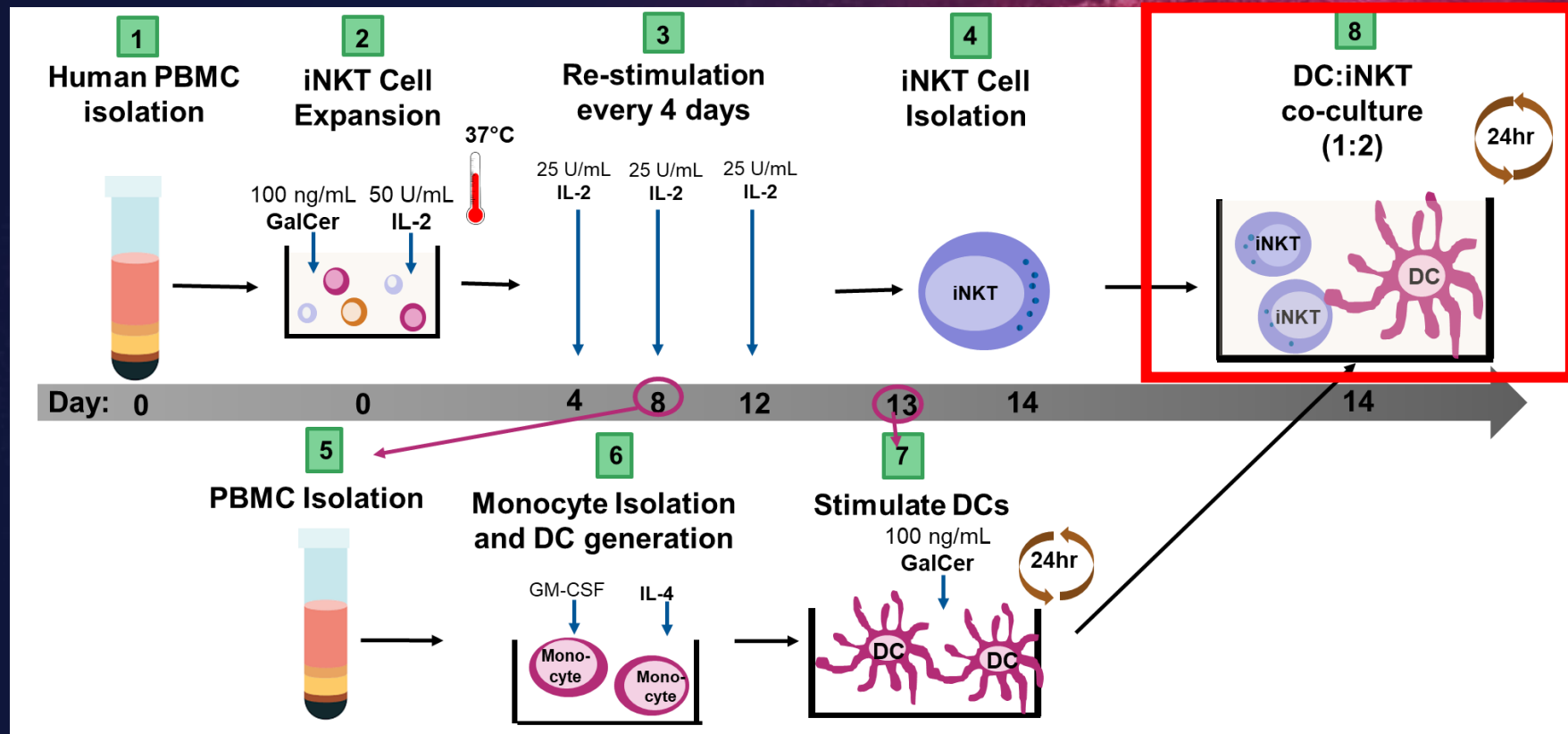
C.



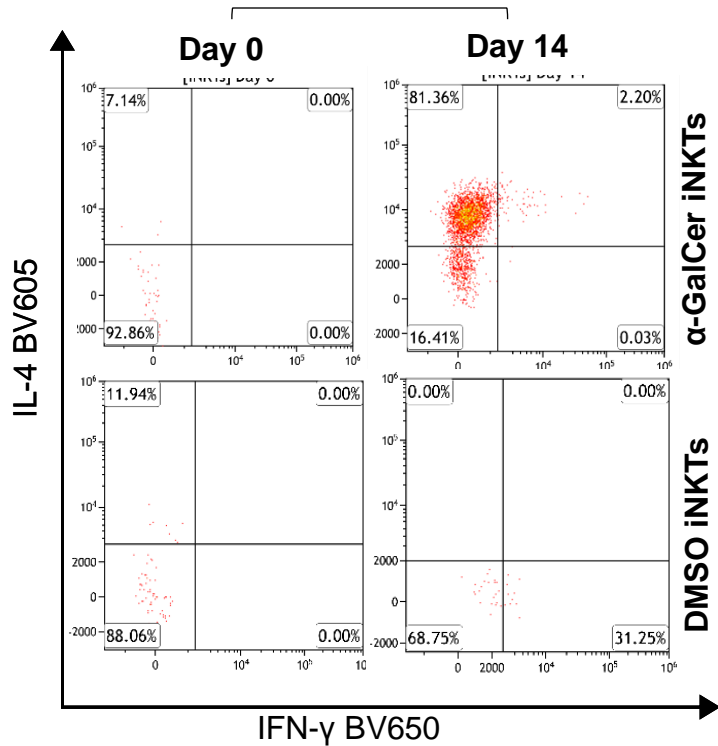
- Immature DCs were successfully generated and were matured using the standard method of LPS stimulation.
- The glycolipid, α -GalCer, did not mature DCs.
- CD1d expression not up regulated by α -GalCer.

Results

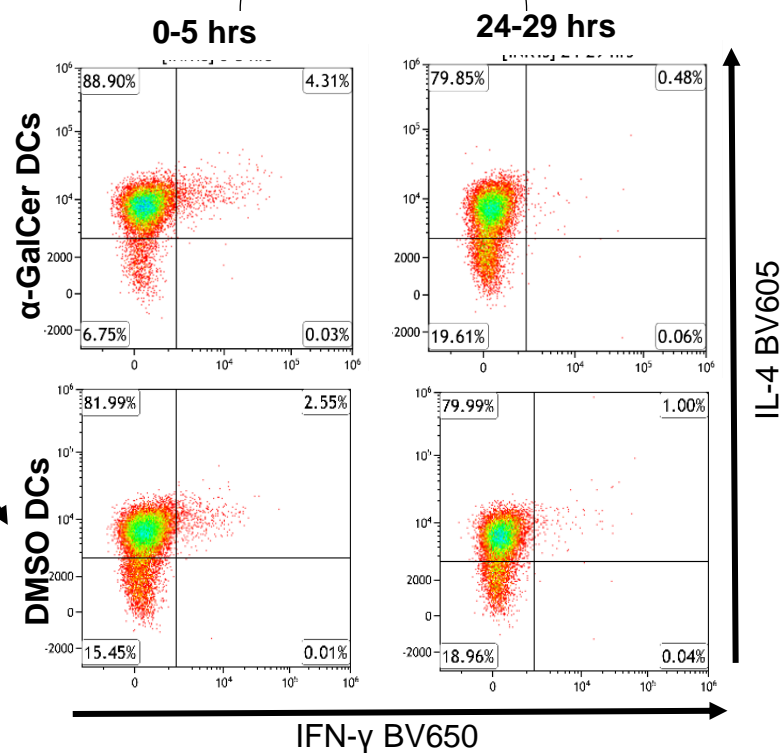
Co-culture cytokine release



iNKT Expansion



DC: iNKT Co-culture



- At **0-5 hours** of iNKT cell co-culture with α-GalCer-pulsed DCs, the number of iNKT cells secreting IL-4 increased by **7.26%**. The number of iNKT cells secreting both IL-4 and IFN-γ increased by **2.11%**.
- The iNKT cells cultured with DMSO-pulsed DCs only showed an increase of **0.63%** iNKT cells secreting IL-4, and **0.35%** of iNKT cells secreting both IL-4 and IFN-γ.
- The secretion of iNKT cell cytokines reduced by 24 hours of co-culture.
- This suggests that the majority of cytokine release occurs rapidly after activation by the DCs, within the first few hours.

Conclusion



- Using the lipid α -GalCer, a model system was developed and optimised to measure iNKT cytokine responses.
- α -GalCer, increased Th2 cytokine secretion of iNKT cells within 5 hours of stimulation.
- This system can be applied using lipids associated to food allergens, to investigate whether they also increase Th2 cytokine secretion, shifting to allergic sensitisation.



1. Blood will be isolated from non-allergic and peanut or soy allergic patients, and this co-culture experiment will be replicated, replacing the lipid a-GalCer with peanut or soy lipids.
2. Lipids used in the experiment will be isolated from peanut seeds, using the Folch. Method and high-performance thin-layer chromatography (HPTLC) for lipid class analysis
3. Total and allergen-specific IgE levels will be measured using an ELISA, for healthy and allergic participant plasma samples.

**This assay development will then allow the main PhD hypothesis to be tested:
Peanut lipids and soy lipids can influence allergic sensitisation to peanut and soy
allergens.**



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Thanks for listening!

Any Questions?

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