# A diversity of novel type-2 innate lymphoid cell subpopulations revealed during tumour expansion

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## Background

- Current **cancer immunotherapeutics** (e.g. CAR T cell) lack efficacy in solid tumours and are costly, necessitating expansion to other immune cells[1].
- A form of **metastatic tumor immune escape involves downregulation of interleukin-33** (IL-33), a potent activator of **type 2 innate lymphoid cells (ILC2)**.
- Innate immune system is crucial in orchestrating immune responses to cancers and **ILC2s play a central role in directing other key players in immune surveillance through multiple immune functions**[2,3].
- Our lab has found that ILC2s are important in controlling tumour progression. **ILC2s isolated from lungs and tumours display variable efficacies against tumour growth** (Figure 2).

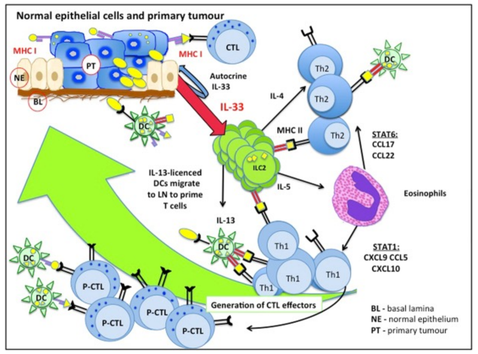



**Figure 1: IL-33-expressing tumour environment stimulates the development of ILC2 cells,** triggering both innate and adaptive immune responses in various mechanisms.

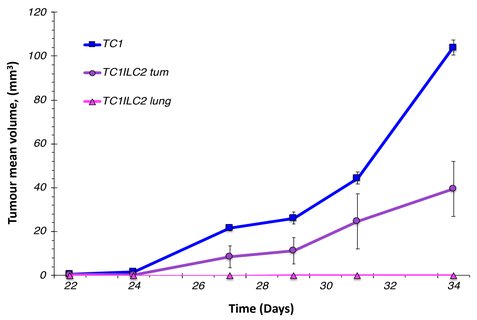



**Figure 2**: **Adoptive transfer of ILC2s reduces tumour burden**. Adoptive transfer of ILC2s into mice bearing TC1 tumour transplants resulted in a significant decrease in overall tumour volume and reduced disease severity.

## Hypothesis/Objective

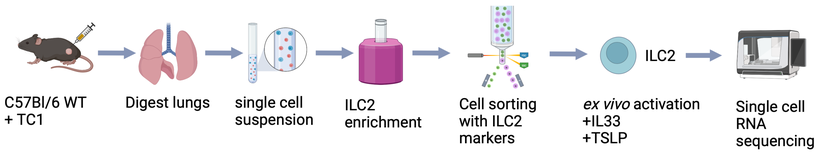
- **ILC2s from different tissues have differential efficacies in controlling tumour growth due to the heterogeneity of this immune cell.**
- **Elucidating the heterogeneity of ILC2s and their potential multi-model immune functions towards anti-tumour immunity.**

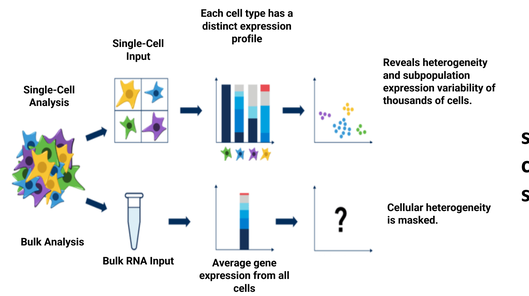
## References

**1.** Bagley, S. J. & O'Rourke, D. M. Clinical investigation of CAR T cells for solid tumors: Lessons learned and future directions. Pharmacol Ther 205, 107419 (2020).
**2.** de Lucia Finkel, P., Xia, W. & Jefferies, W. A. Beyond Unconventional: What Do We Really Know about Group 2 Innate Lymphoid Cells? J Immunol 206, 1409-1417 (2021).
**3.** Saranchova I, Han J, Zaman R, Arora H, Huang H, Fenninger F, et al. Type 2 Innate Lymphocytes Actuate Immunity Against Tumours and Limit Cancer Metastasis. Sci Rep. 2018 Feb 13;8:2924.
**4.** Xia, C.W., Saranchova, I., Finkel, P.L. *et al.* A diversity of novel type-2 innate lymphoid cell subpopulations revealed during tumour expansion. *Commun Biol* **7**, 12 (2024). https://doi.org/10.1038/s42003-023-05536-0

## Methods

### Single-cell RNA sequencing approach




**scRNA-seq reveals tissue and cellular heterogeneity on a single-cell level**

## Results

### Subpopulations of tumour-associated ILC2s

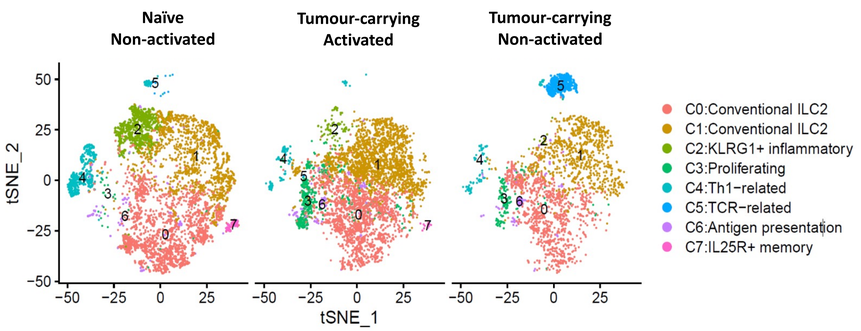



**Figure 3:** t-distributed stochastic neighbor embedding (tSNE) plot clustering of single cell RNA sequencing (scRNAseq) data of naïve non-activated, tumour-carrying IL-33 activated and non-activated lung isolated ILC2 subsets using the Seurat R package. Subpopulations are defined by pre-set genes-of-interest (Figure 4a).

**Table 1: Dynamic changes of lung ILC2 subpopulation frequencies under different conditions.** ILC2s are a heterogeneous and dynamic immune cell population.

| Cluster | ILC2 Subtype | Cluster frequency % (cell count) | | |
|---|---|---|---|---|
| | | Naïve Non-activated | Tumour-carrying Activated | Tumour-carrying Non-activated |
| 0 | Conventional ILC2 | 38 (2321) | 35 (2029) | 36 (1117) |
| 1 | conventional ILC2 | 27 (1616) | 46 (2679) | 3 (923) |
| 2 | KLRG1+ inflammatory | 19 (1140) | 2 (117) | 0 (10) |
| 3 | Proliferation | 1 (36) | 13 (742) | 5 (169) |
| 4 | Th1-related | 11 (674) | 2 (132) | 3 (92) |
| 5 | TCR-related | 0 (13) | 0 (1) | 22 (670) |
| 6 | Antigen presentation | 2 (151) | 2 (126) | 4 (117) |
| 7 | IL25R+ memory | 2 (145) | 0 (14) | 0 (0) |

Highest ⬇ Lowest

## Results

### Subpopulations of ILC2s are enriched with different molecular signatures indicating diverse cellular functions

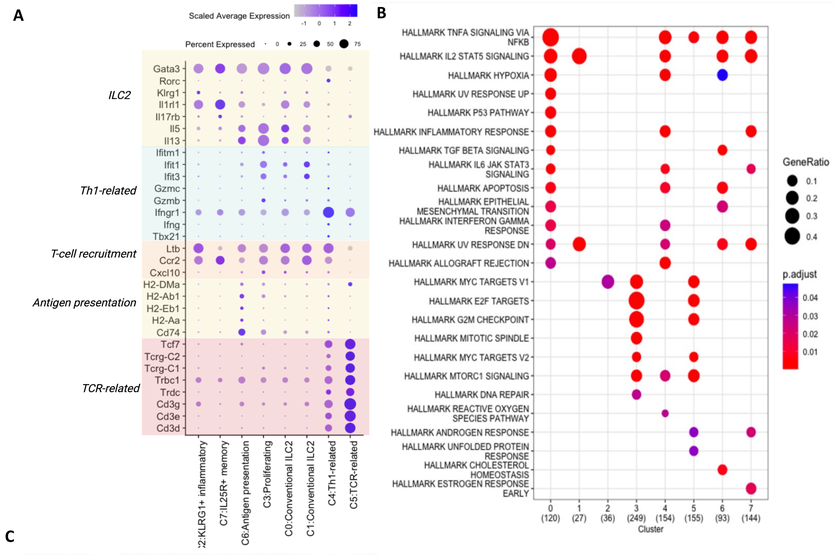



**Figure 4: A**) Expression of selected ILC2 markers-of-interest (GOIs) are differentially expressed by different clusters. **B**) Dotplot indicates different gene sets being enriched in each subpopulation/cluster. **C**) Validation of ILC2s in mouse lung tissue using triple immunofluorescence labelling of GATA3 (red), ICOS (green) and CD3 (white).

## Conclusions

- **Lung ILC2s are a heterogenous**, with subtypes involved in antigen presentation, T cell recruitment and type1-immune responses.[4]
- **Tumour presence and IL-33 activation result in heightened pro-inflammatory type1-like genotype** while maintaining type 2 identity of ILC2s.
- Marker/function validations of subtypes are underway to further elucidate the functional heterogeneity of ILC2s.
- **Overall, this work improves our understanding of cancer surveillance with eventual translation to ILC2 cancer immunotherapy.**

## Funding

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