**RORt+ innate lymphoid cells promote lymph node metastasis in breast cancers.**

Sheeba Irshad1\*, Fabian Flores-Borja1,2\*, Katherine Lawler2,3, James Monypenny2, Rachel Evans2, Victoria Male1, Peter Gordon1,2, Anthony Cheung2, Patrycja Gazinska1, Farzana Noor1, Felix Wong2, Anita Grigoriadis1, Gilbert O Fruhwirth2, 10, Paul R Barber4, Natalie Woodman5, Dominic Patel11, Manuel Rodriguez-Justo11, Julie Owen5, Stewart Martin6, Sarah E Pinder5,7, Cheryl E. Gillett5,7, Simon P Poland2, Simon Ameer-Beg2, Frank McCaughan8,9, Leo M. Carlin10, Uzma Hasan11, David R Withers12, Peter Lane12, Borivoj Vojnovic4, Sergio A Quezada13, Paul Ellis14, Andrew Tutt1, 15 and Tony Ng1,2,13.

1Breast Cancer Now Research Unit,KCL, London, SE1 9RT.

2Richard Dimbleby Department of Cancer Research, Randall Division & Division of Cancer Studies, KCL, Guy’s Medical School Campus, SE1 1ULK.

3 Institute for Mathematical and Molecular Biomedicine, KCL, Hodgkin Building, Guy’s Medical School Campus, SE1 1UL.

4Gray Institute for Radiation Oncology & Biology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ.

5King’s Health Partners Cancer Biobank, KCL, Guy’s Hospital, London SE1 9RT.

6School of Medicine, Division of Cancer and Stem Cells, Department of Clinical Oncology, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham NG5 1PB

7Research Oncology, Division of Cancer Studies, KCL, 3rd Floor, Bermondsey Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT.

8 Department of Asthma, Allergy, and Lung Biology, KCL, Guy's Hospital, Great Maze Pond, London, SE1 9RT.

9Department of Biochemistry, University of Cambridge, Cambridge

10 Leukocyte Dynamics Group, Beatson Advanced Imaging Resource, CRUK Beatson Institute, Glasgow.

11International Center for Infectiology Research, University of Lyon, Lyon 69007, France; Inserm, U1111, Lyon 69007, France; Ecole Normale Supérieure de Lyon, Lyon 69007, France; Université Claude Bernard Lyon 1, Centre International de Recherche en Infectiologie, Lyon 69100, France; Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5308, Lyon 69007, France; Oncovirus et l'immunité innée, Hospices Civils de Lyon Sud, Pierre Benite, 69495 France; uzma.hasan@inserm.fr.

12MRC Centre for Immune Regulation, Institute for Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT.

13 UCL Cancer Institute, Paul O'Gorman Building, University College London, London WC1E 6DD, UK

14 Department of Medical Oncology, Guy’s and St Thomas Foundation Trust, London SE1 9RT

15 ICR, Breast Cancer Now Research Unit,Toby Robins Research Centre, London

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**Supplemental Inventory**

1. **Supplemental Figures, Tables and Videos**

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**2. Supplemental Experimental Procedures**

Table S2: List of antibodies used

Table S3: Sequence of oligonucleotides used

**Figure S1, Related to Figure 1**

**A**) FACS sorted Lin-CD127+CD90.2+NKP46- gated cells in the primary tumor express additional markers CCR6, CD4 and RORγT that define LTi/ILC3. **B**) Immunofluorescence of unsorted CD11c-depleted splenocytes. An ILC3 cell (white arrow) is seen amongst CD3+ cells. Bottom panel demonstrated that majority of the sorted cells express the nuclear transcription factor RORT.

Figure S2, Related to Figure 2

**A:** Protein levels of CXCL13 and CCL21 in conditioned media (CM) obtained from the bone marrow derived MSC cell line (HS-5) compared to non-conditioned media were measured by ELISA. MSC cells seeded at densities of either 6x103 or 12x103 cells per well are shown above. Data represent means of three independent experiments ± SEM. **B & C:** ELISA quantification of CXCL13 and CCL21 chemokine levels in the conditioned media from the breast cancer cell line 4T1.2 cell line is shown. Positive control = murine stromal cell line (ST2s); Negative control = media alone. Asterisks represent the p-values when comparing to the control groups (one-way ANOVA; \*\* p≤0.01).

**Figure S3, Related to Figure 2**

**A:** ILC3 cells do not proliferate when co-cultured with human MSCs. FACS-sorted ILC3 cells were labelled with an eFluor450 cell tracker dye and co-cultured with human HS-5 MSC cell line (ratio 10:1, ILC3:MSC) for 48h. As proliferation positive control, cell-tracker dye-labelled splenocytes were stimulated with plate-bound anti-CD3 (1 g/ml) and soluble anti-CD28 (2 g/ml) antibodies. At the end of the co-culture cells were stained with a viability dye and analysed by flow cytometry. **B and C:** MSC cells were transfected with non targeting (NT) control or siRNAs specific for CXCL13 or CCL21 (CXCL13 siRNA: si-20725, si-20726, si-20727 or CCL21 siRNA: si-12605, si-12606, si-12607 etc respectively). Cell culture supernatents were analyzed by ELISA at 48hours (\* p≤0.05, \*\* p≤0.01, paired t-test).

**Figure S4, Related to Figure 4**

**A**: CXCR5 expression on MSC cells. The histograms show the expression of CXCR5 on HS-5 human MSC cell line and human peripheral blood B-cells (positive control) as compared to control isotype antibody. The graph on the left shows cumulative data from three different experiments.

**Figure S5, Related to Figure 5**

Breast cancer tissue with prominent tertiary lymphoid follicle formation. Tumor infiltrating lymphocytes are not only scattered throughout the stroma and interspersed between tumor cells; they also cluster in aggregates resembling tertiary lymphoid tissue with distinct compartmentalization between a T and a B cell zone. **A:** H&E histopathological images show (**A**): Low power view of a breast cancer tumour section and (**B**): High power view allows identification of dense areas of lymphoid cell aggregates within the human breast cancer microenvironment (red circles). **C**: Immunofluorescence for CD3 (membrane or cytoplasmic blue staining) identifies the outer T-zone of these lymphoid structures. **D**: Identification of a RORT+CD127+CD3- ILC3 cells (white arrow) (RORT+, green nuclear; CD127+, red perinuclear) within the TLS. TZ=T zone. Red squares represent areas of interest.

**Figure S6, Related to Figure 5**

**Expression of lymphoid chemokine and chemokine receptor genes in breast cancer datasets.** Heat maps display gene-standardised expression for each breast cancer data set. Columns (samples) are ordered by increasing expression score (mean of gene standardised expression values of the listed genes) and displayed in blocks representing score quintiles (left-right, lowest to highest expression score). PAM50 assignments are depicted above. Bar plots show the distribution of intrinsic subtype assignments within each expressionquintile. Relative enrichment for the basal-like subtype (red) amongst samples withhigher expression scores is observed in multiple independent data sets.

**Table S1, Related to Figure 5**

**Table S1:** Clinico-pathological characteristics for the METABRIC sample.

1. Guy's METABRIC gene expression data set. Poor quality arrays were filtered out, leaving 234 out of a possible 250 samples for analysis for lymphoid gene expression analysis.
2. Guy's METABRIC gene expression data set stained for ILC3 density data (n=59).

**Video S1, Related to Figure 2**

Time lapse microscopy experiment of sorted NKp46-ILC3 cells (CD3-, CD11c-, B220, NKp46-, CD127+, CD90.2+) co-cultured with MSC cells as described in Figure 2B. Low magnification (×10) video shows the general clustering pattern of NKp46-ILC3 cells around the MSC cells over a duration of 10 hours. Experimental conditions were as described in Figure 2B legend and in more detail in Experimental Procedures.

**Video S2, Related to Figure 2**

Time lapse microscopy experiment of sorted NKp46-ILC3 cells (CD3-, CD11c-, B220-, NKp46-, CD127+, CD90.2+) co-cultured with MSC cells as described in Figure 2B. High magnification (×40) video shows the prolonged interaction of NKp46-ILC3 and MSC cells upon contact. Experimental conditions were as described in Figure 2B legend and in more detailed in Experimental Procedures.