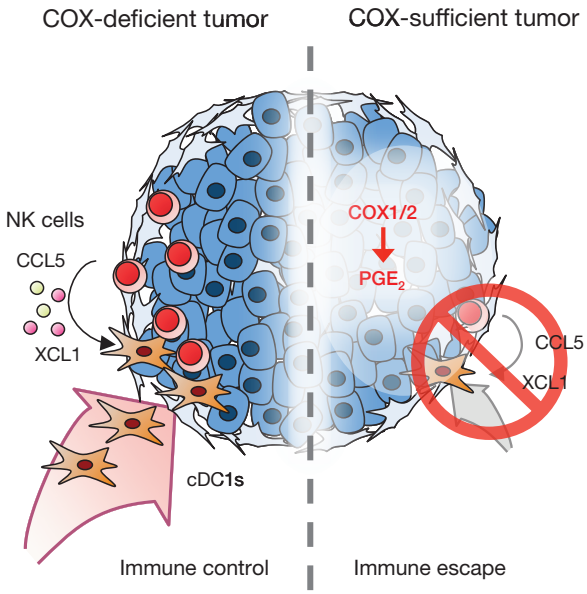
Tracking intratumoral NK cell function by flow cytometry

Böttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, Rogers NC, Sahai E, Zelenay S, Reis e Sousa C (2018) NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* 172:1022–1037.

Dramatic advances in immuno-oncology (I-O) research have led to the development of cancer immunotherapies that target the host immune system to attack tumor cells. These immunotherapies allow the successful treatment of significant fractions of cancer patients who do not respond to gold-standard "designer" cytotoxic therapies, which are tailored to target cancer based on specific molecular and pathological criteria [1]. I-O therapeutic approaches target cancer-associated immune responses to neutralize tumor-promoting chronic inflammation, block immunosuppressive mediators in tumors, or stimulate the antitumor activity of innate and adaptive immune cells such as antigen-specific cytotoxic T cells (CTLs) [2,3]. While powerful, the effectiveness of these strategies is limited by the nature of cancer cells, which can evade antitumor immunity and become invisible to the immune system through a variety of mechanisms [4].

Effective antitumor CTL responses are a key component of immune system–mediated control of cancer. Like all adaptive immune responses, antitumor CTL responses are dependent on conventional dendritic cells (cDCs), which are specialized in the presentation of tumor antigens and secrete cytokines that regulate CTL survival and effector function within the tumor. Conventional type 1 dendritic cells (cDC1s), in particular, excel at taking up dead tumor cells and transporting tumor antigens to tumor-draining lymph nodes where they cross-prime antitumor CTL responses [5,6]. They also play a role within the tumor itself, where they attract T cells and can restimulate tumor-infiltrating T cells [7,8]. Many tumors build resistance to CTL responses by secreting the prostanoid prostaglandin E2 (PGE_2), which inhibits the recruitment of intratumoral cDC1s and the development of effective antitumor CTL responses, leading to tumor growth. The ablation of the PGE_2-producing cyclo-oxygenase genes *Ptgs1* and *Ptgs2*, in turn, restores cDC1-dependent CTL-mediated tumor control [9].

Böttcher and colleagues recently reported their use of the Invitrogen™ PrimeFlow™ RNA Assay (**thermofisher.com/primeflow**) to identify a key role of intratumoral NK cells in the development of antitumor $CD8^+$ CTL responses by secreting XCL1 and CCL5 and recruiting CTL-priming cDC1s into the tumor microenvironment in mouse tumors [10] (Figure 1). They demonstrate that PGE_2-mediated tumor suppression of anticancer immunity works by diminishing NK cell survival and chemokine production and downregulating cDC1 responsiveness to XCL1 and CCL5 (Figure 1). →



Highlights

- NK cells recruit cDC1s into the tumor microenvironment
- cDC1 recruitment depends on NK cell–derived chemokines CCL5 and XCL1
- The NK cell/chemokine/cDC1 axis is associated with cancer patient survival
- Tumor-derived PGE_2 impairs NK cells and cDC1s, resulting in cancer immune evasion

Figure 1. Graphical abstract from "NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control." Reprinted with permission, from Böttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, Rogers NC, Sahai E, Zelenay S, Reis e Sousa C (2018) *Cell* 172:1022–1037, and under the Creative Commons Attribution 4.0 International License (**creativecommons.org/licenses/by/4.0**).

Characterization of XCL1 mRNA–expressing intratumoral NK cells

In their publication, Böttcher et al. characterize XCL1-producing intratumoral NK cells using PrimeFlow Type 1 (Alexa Fluor™ 647) oligonucleotide probe sets specific for XCL1 mRNA. This analysis of intracellular XCL1 mRNA transcripts was done in combination with extensive protein immunophenotyping by flow cytometry.

They also examined tumor-infiltrating leukocyte (TIL) extracts from PGE_2-sufficient and -deficient (*Ptgs1/Ptgs2*$^{-/-}$) tumors in mice and found that the presence of PGE_2 led to decreased intratumoral NK cell presence and inhibited their ability to produce cDC1 chemoattractants XCL1 and CCL5. This, in turn, reduced CTL numbers in the tumor, resulting in increased tumor mass. These results were confirmed by inmmunofluorescence microscopy staining of tumor tissue sections and also by multiplex ELISA assays.

The authors further explored tumor gene expression data from The Cancer Genome Atlas (TCGA) and found strong evidence that a similar NK cell/chemokine axis exists in multiple types of human cancers and correlates positively with patient survival, suggesting this mechanism bears direct clinical relevance.

Future directions for I-O therapies

The development of increasingly specific and effective I-O therapies requires progressively deeper understanding of the cellular and biochemical mechanisms of immunological control of cancer, and of how cancers manage to evade surveillance by immune cells such as NK cells and cDC1s. Achieving this deeper understanding will entail meticulous mapping of immune cell function within the tumor microenvironment (TME), in addition to tracking how immune cell networks behave and interact with tumor cells during the cycle of tumor progression, remission during treatment, development of treatment resistance, and resurgence. ■

References

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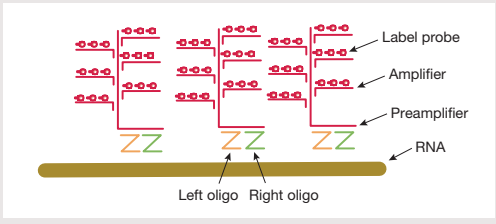
More about the PrimeFlow RNA assay

With the Invitrogen™ PrimeFlow™ RNA Assay Kit, researchers can reveal the dynamics of RNA transcription together with protein expression patterns at the single-cell level by multicolor flow cytometry. The PrimeFlow RNA assay employs fluorescence *in situ* hybridization (FISH) with branched DNA (bDNA) signal amplification for the simultaneous detection of up to 4 RNA targets, and it can be used in combination with immunolabeling of both cell-surface and intracellular proteins using fluorophore-conjugated antibodies.

In the PrimeFlow RNA assay workflow, cells are first labeled with cell-surface antibodies, fixed and permeabilized, and then labeled with intracellular antibodies. Next, the cells are hybridized with oligonucleotide probes specific for the RNA targets. Hybridized targets are detected after bDNA amplification, which is achieved through sequential hybridization steps with preamplifiers, amplifiers, and fluorophore-conjugated label probes. A fully assembled amplification "tree" has 400 label probe–binding sites, and can produce >8,000-fold signal amplification.

With target-specific probe sets, the PrimeFlow RNA assay can be used to detect miRNA, lncRNA, and mRNA, as well as viral RNA and telomere DNA. The PrimeFlow RNA Assay Kit provides reagents for detecting up to 4 RNA transcripts in mammalian cells optionally labeled with antibodies that recognize cell-surface or intracellular proteins. For more information, including catalog probe sets and ordering guidelines, visit **thermofisher.com/primeflow**.



Branched DNA (bDNA) amplification scheme used in the PrimeFlow RNA assay.

Product	Quantity	Cat. No.
PrimeFlow™ RNA Assay Kit	40 tests	88-18005-204
	100 tests	88-18005-210