IFN-gamma is Central to Both Immunogenic and Tolerogenic Properties of Dendritic Cells After IL-12 and GM-CSF Microsphere Treatment

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Previous Data:

**Tumor draining lymph node (TDLN)**

- Activation of effector and memory CD8+ T-cells in TDLNs
- Reduction of Tregs
- Infiltration of NK cells
- Post-treatment T-reg rebound

**GM-CSF and IL-12 Microsphere Rx**
Background

- **Dendritic Cells (DCs)**
  - The most potent antigen presenting cell – master regulator of the immune response
  - Can prime in an immunogenic or tolerogenic fashion

How does GM-CSF and IL-12 microsphere treatment affect DCs?
DC numbers increase in tumor and tumor draining lymph nodes after treatment.

Absolute DC numbers in the tumor

Absolute DC numbers in the TDLN post-Rx
DCs post-treatment upregulate CCR7 and CD86

Gated on CD45+, CD11c+, MHCII+ (DCs)

Pre-treatment  Day 1 post-Rx

%CD86 high DCs

**CCR7 MFI**

% CD86 high DCs

%CD86+ DCs
DCs become immunogenic after treatment, followed by tolerogenicity.

What is the cause of the poor DC priming ability by Day 7 post-treatment?
IDO is upregulated in DCs post-treatment

Intracellular Staining for IDO

Primary antibody with isotype control secondary antibody:

Intracellular IDO staining of D7Rx TDLN sample:

MFI = 91.8

Isotype control primary antibody with secondary antibody:

Intracellular IDO staining of untreated TDLN sample:

MFI = 32.3

DC expression of IDO

*
IDO mediates post-treatment DC tolerogenicity.

Addition of D1MT restores priming ability to D7Rx DC, but not pre-treatment DCs.
Requires IFN-gamma

- Effector memory CD8+ T-cell activation
- Reduction in T-reg numbers
- NK cell activation
- *De novo* CD8+ T-cell priming in TDLN

Is IFN-gamma required for treatment mediated affects on DCs?
IFN-gamma drives DC immunogenicity

**DC numbers in TDLN**

- **Day 0**: WT (black) = 2, IFN-gamma k/o (gray) = 1
- **Day 2**: WT (black) = 6, IFN-gamma k/o (gray) = 4

**% CD86 high**

- **Day 0**: WT (black) = 20, IFN-gamma k/o (gray) = 15
- **Day 2**: WT (black) = 50, IFN-gamma k/o (gray) = 35

**DC priming ability**

- **D0**: WT (black) = 40, IFN-gamma k/o (gray) = 30
- **D2**: WT (black) = 70, IFN-gamma k/o (gray) = 50
- **D7**: WT (black) = 30, IFN-gamma k/o (gray) = 20

* = statistically significant difference between WT and IFN-gamma k/o conditions.
IFN-gamma drives DC regulation via IDO

IDO Upregulation in DCs requires IFNgamma

- **WT**
- **IFNgamma k/o**

Day 0: 
- WT MFI: 30
- IFNgamma k/o MFI: 40

Day 2: 
- WT MFI: 30
- IFNgamma k/o MFI: 40

Day 7: 
- WT MFI: 90
- IFNgamma k/o MFI: 90

* indicates a significant difference.
Addition of D1MT to microsphere treatment results in complete regression of tumors.

Central Role of IFNγ–Indoleamine 2,3-Dioxygenase Axis in Regulation of Interleukin-12–Mediated Antitumor Immunity. Tao Gu et al. Cancer Res. 70(1) 129-38.
Summary:

Intratumoral GM-CSF and IL-12 microsphere Rx

- Low DC numbers in tumor and TDLN
- CCR7 and CD86 low
- Poor in vitro priming ability

D0

- DC numbers increase in tumor followed by TDLN
- CCR7 upregulation
- CD86 upregulation
- Superior in vitro priming ability

D2

IFN-gamma

D7

IFN-gamma

- Upregulation of immuno-suppressive IDO
- Loss of superior priming ability
- Reversible by addition of D1MT