

# **TLR2/6 Agonists and IFN $\gamma$ Synergize to Induce Melanoma Cells to Produce T cell-Recruiting Chemokines**

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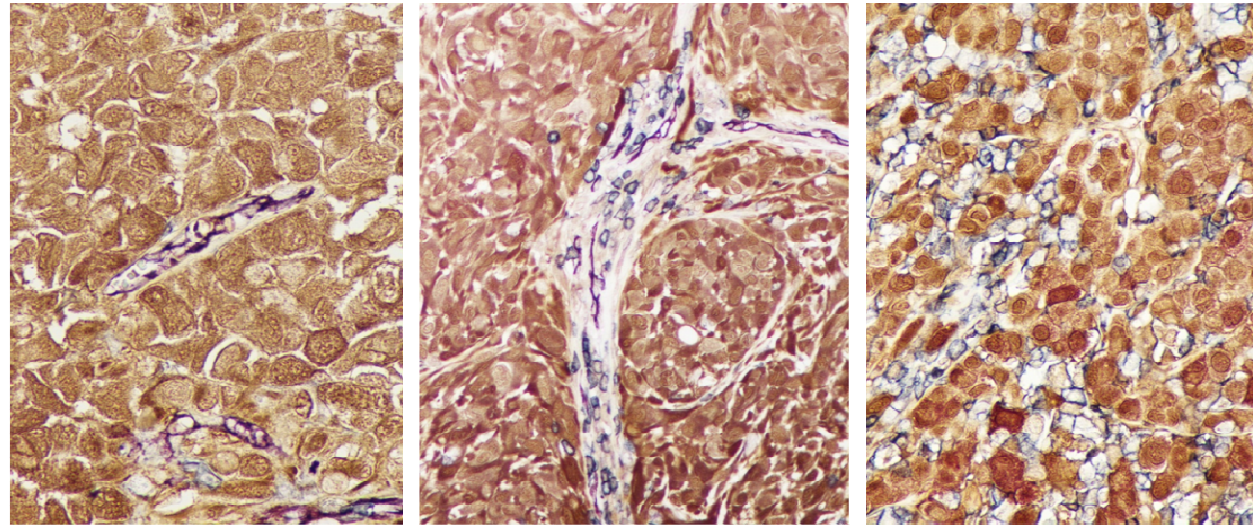
**Presenter Disclosures: No relationships to disclose.**



# Immunotypes in Metastatic Melanoma

S100  
CD31  
CD45

Erdag G *et al.*  
*Cancer Res.* (2012)



**A**

**B**

**C**

**Immune cell infiltrate:** None

Cuffing

Diffuse

**% of Patients:** 29%

63%

8%

**Median survival:** 15 months

23 months

130 months

## *Hypothesis*

***TLR ligation, alone or in combination with IFN $\gamma$ , induces production of T cell-attracting chemokines directly from melanoma cells***



# Methods

## Models:

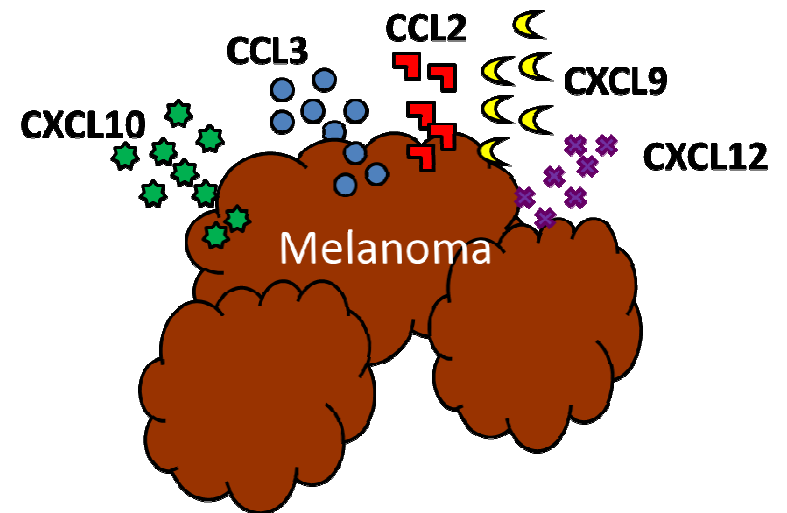
- Patient-derived melanoma cell lines: **VMM1, DM13, DM93 & DM122**
- Freshly resected melanoma specimens

## TLR agonists tested +/- IFN $\gamma$

Imiquimod	25 $\mu\text{g/ml}$
Resiquimod	5 $\mu\text{g/ml}$
CpG	5 $\mu\text{g/ml}$
poly-ICLC	20 $\mu\text{g/ml}$
LPS	10 $\mu\text{g/ml}$
<b>MALP-2</b>	0.1 $\mu\text{g/ml}$
<b>FSL-1</b>	5 $\mu\text{g/ml}$

## Analyzed for chemokines known to recruit T cells:

- CCL2
- CCL3
- CCL4
- CCL5
- CXCL9
- CXCL10
- CXCL12



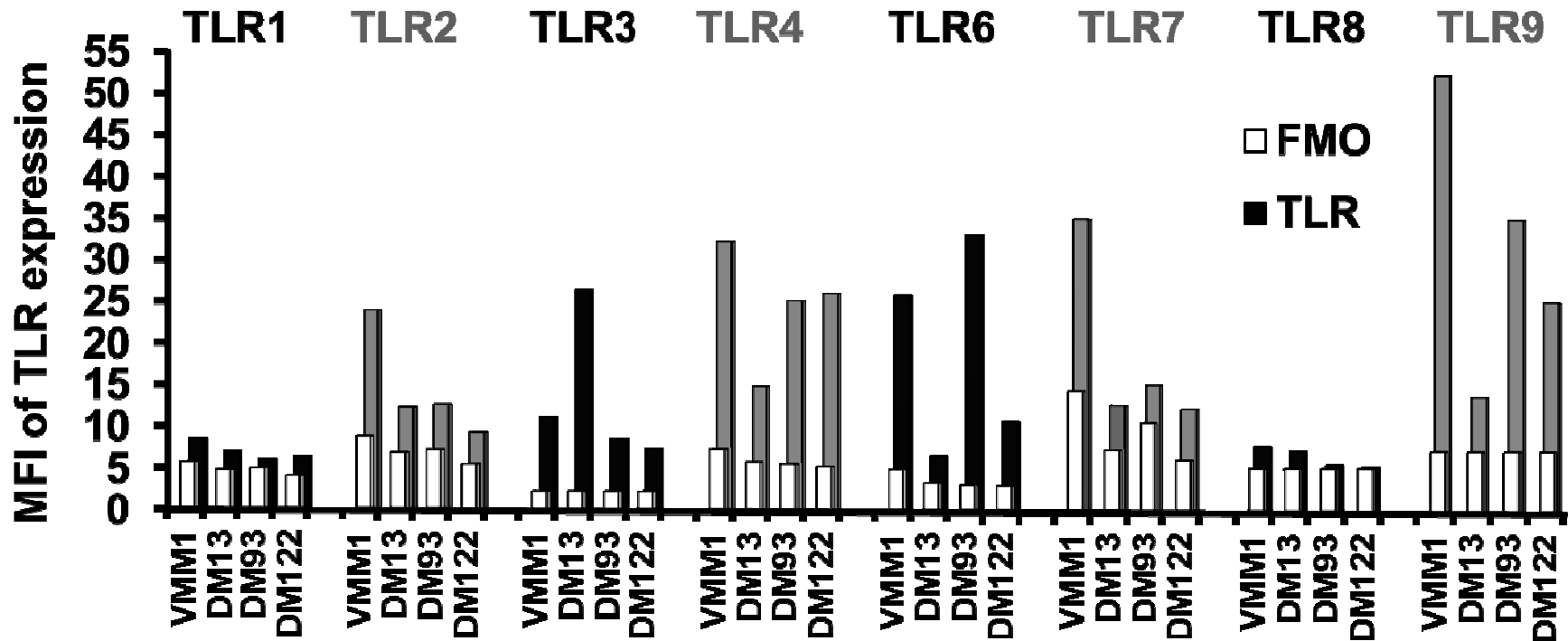
(Harlin H et al. Cancer Res. 2009)



# Melanoma cells express TLRs

**Gene Array:** evidence of TLR expression by human melanoma cells

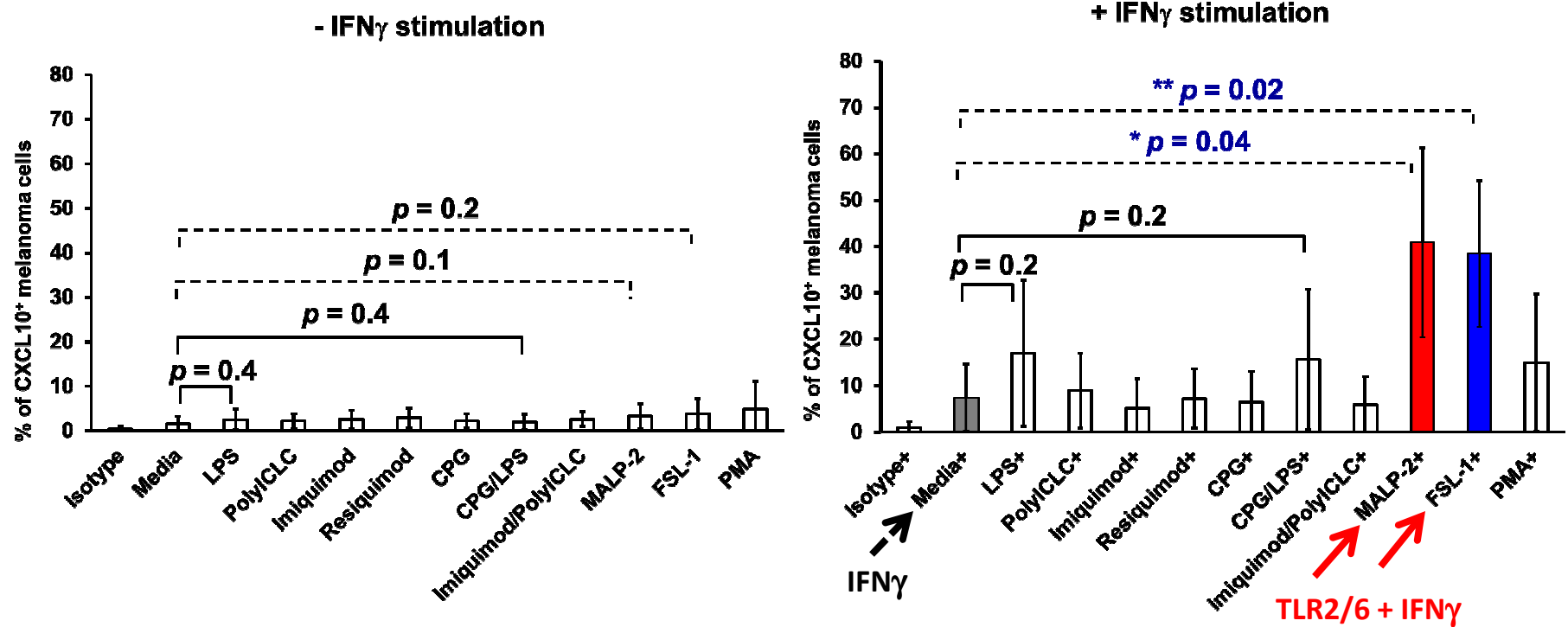
**Flow cytometric analysis**



- CCL2, 4, 5, CXCL9, and 12 were weakly expressed and expression did not increase with TLR agonist stimulation +/- IFN $\gamma$ .
- CCL3 had high basal expression in melanoma cell lines, and was enhanced with TLR 2/6 stimulation (data not shown).



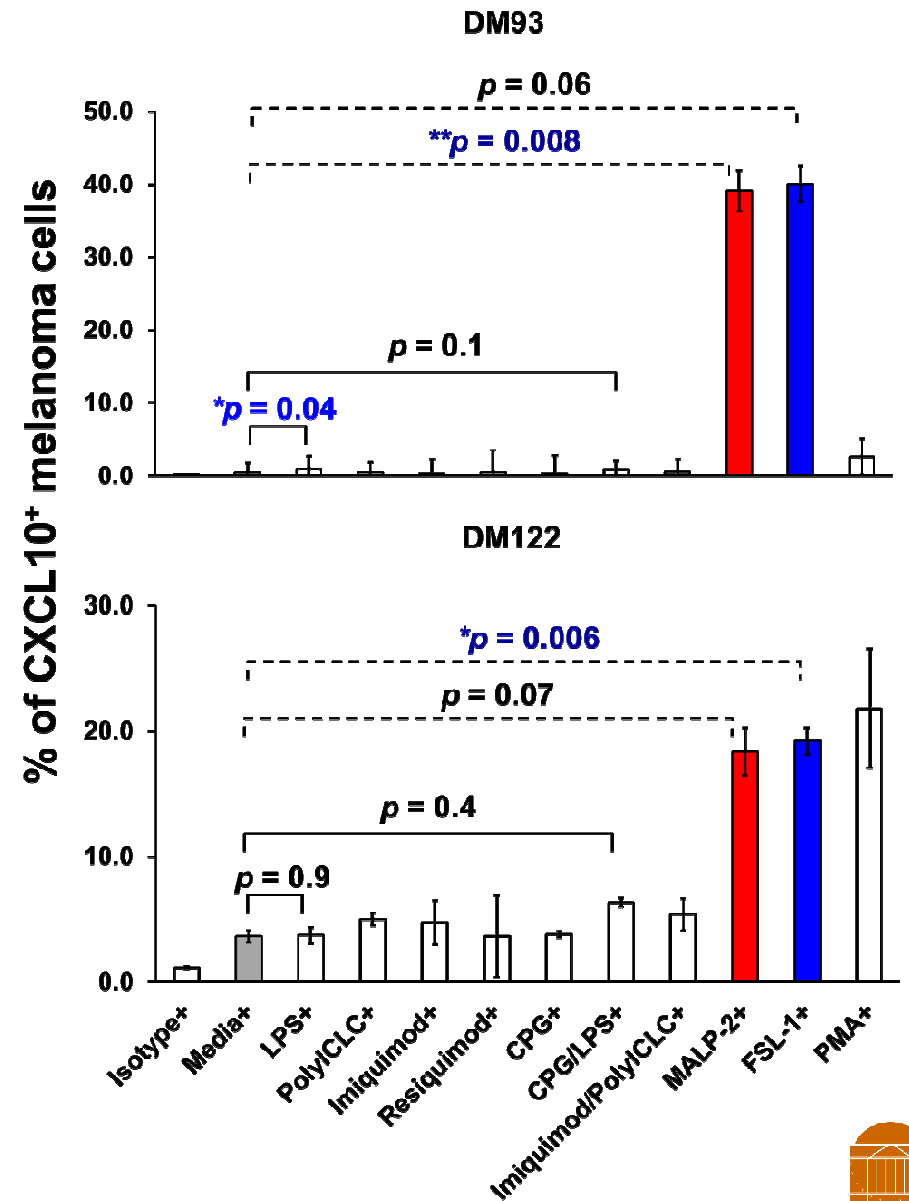
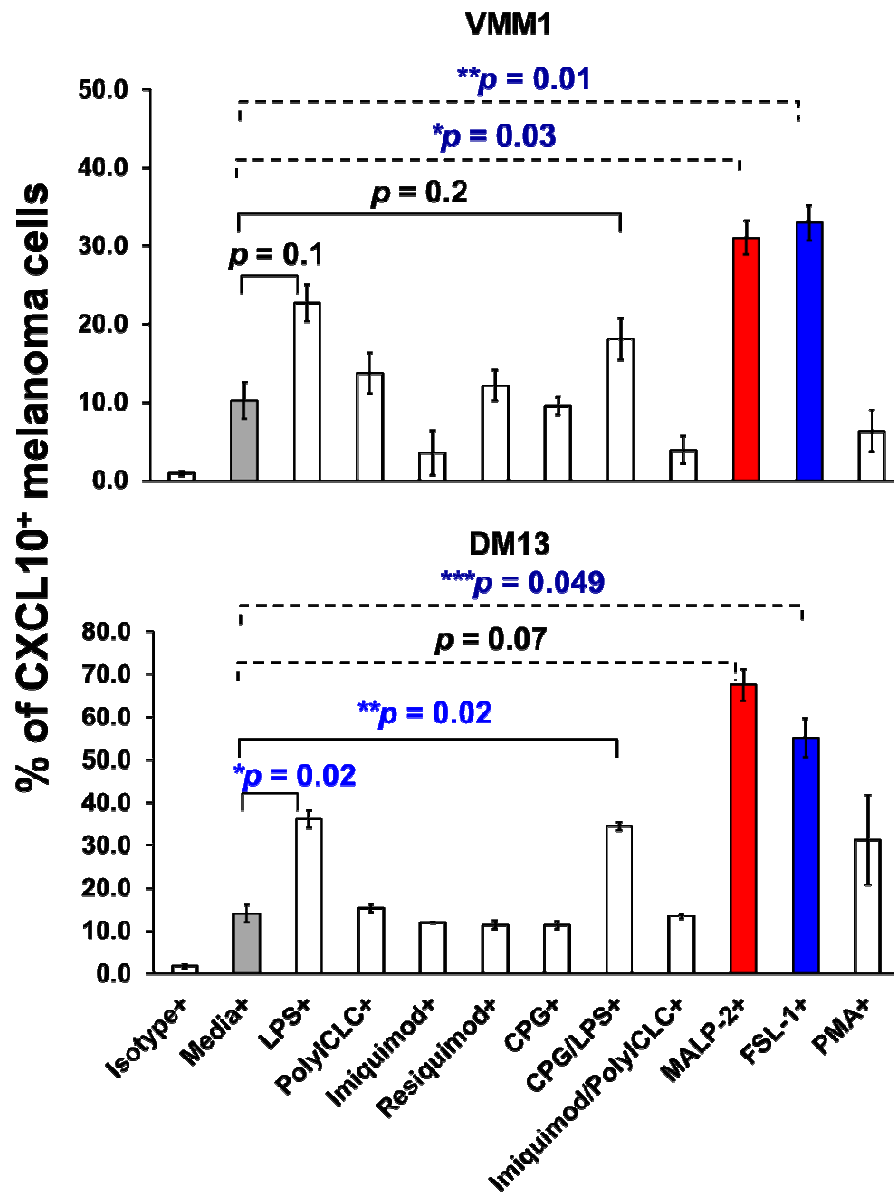
# TLR2/6 agonist and IFN $\gamma$ stimulation synergistically increase the % of melanoma cells that produce CXCL10



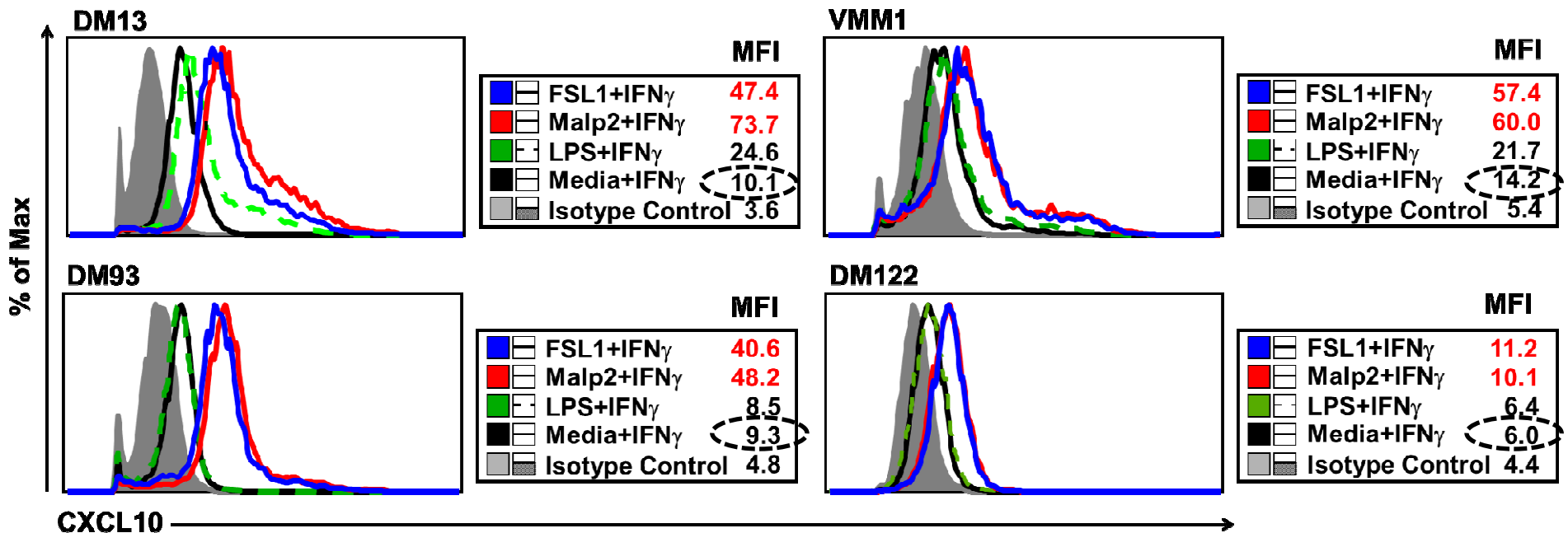
- CXCL10 was induced by IFN $\gamma$ , but production was significantly upregulated when melanoma cells received TLR2/6 agonist + IFN $\gamma$  stimulation.
- High CXCL10 expression in the melanoma microenvironment is associated with better T cell infiltration, tumor control (reduced proliferation, metastasis, angiogenesis), and disease-free survival.



# TLR2/6 agonist and IFN $\gamma$ stimulation synergistically induce CXCL10 production by melanoma cells

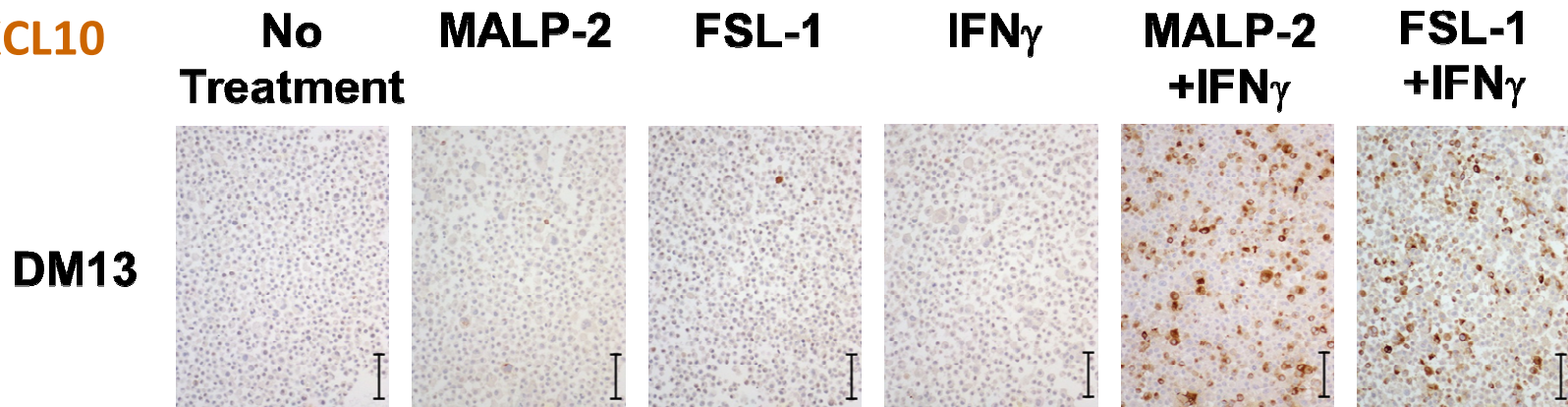


# CXCL10 is synergistically upregulated from TLR2/6 agonist and IFN $\gamma$ stimulated melanoma cells.

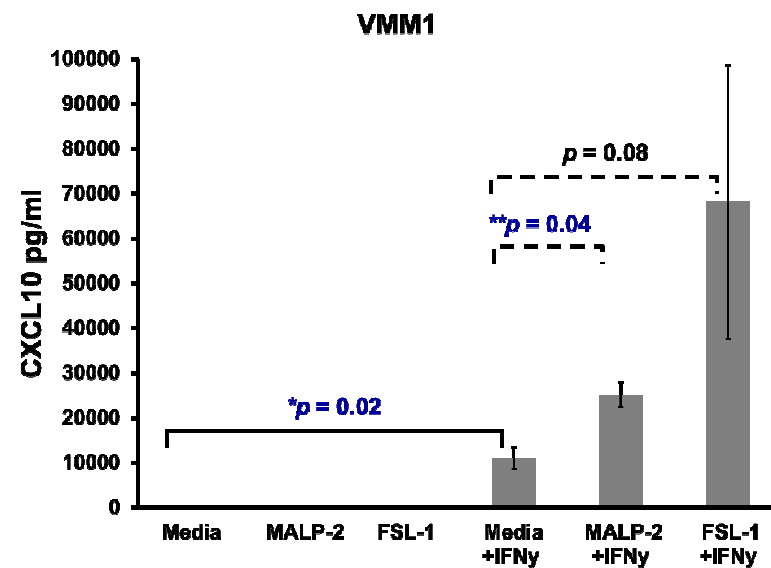
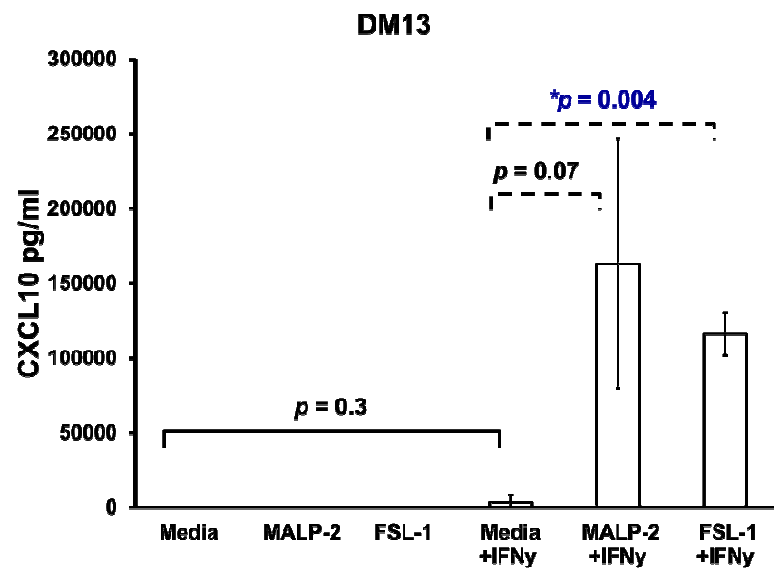


# CXCL10 is synergistically upregulated from TLR2/6 agonist and IFN $\gamma$ stimulated melanoma cells.

## IHC CXCL10



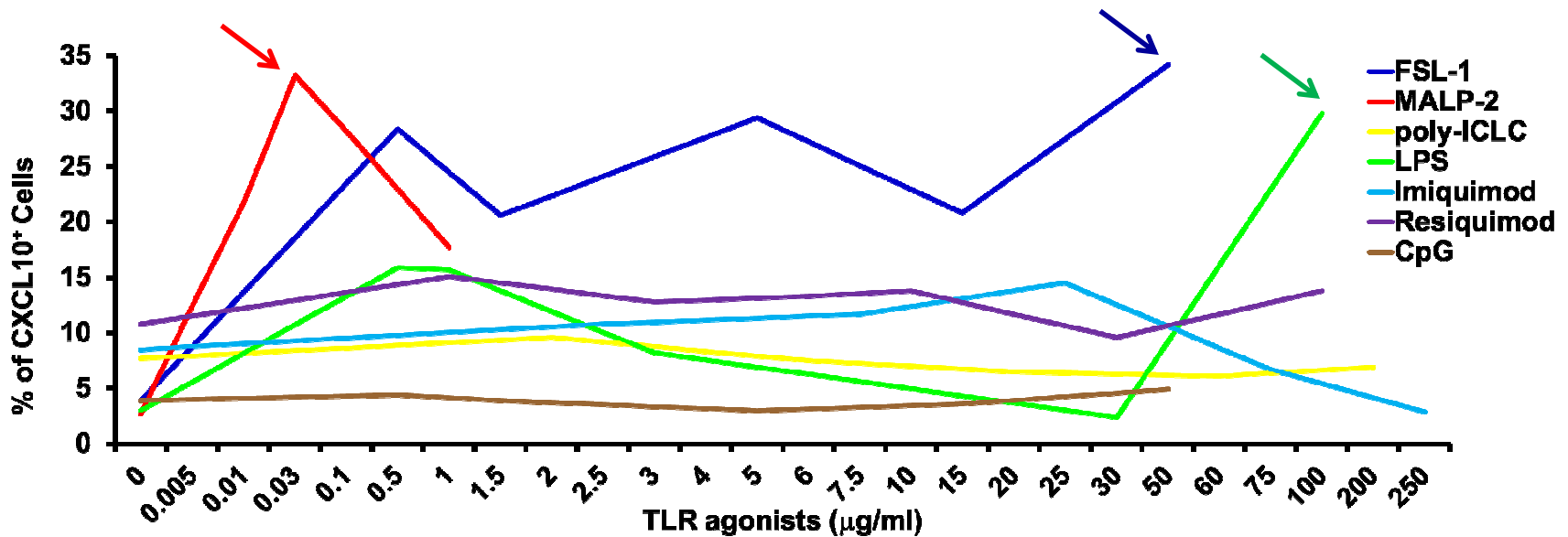
## ELISA assay for CXCL10 production





# TLR2/6 agonists effectively increase CXCL10 production from melanoma cells.

## Dose response assay for CXCL10 production



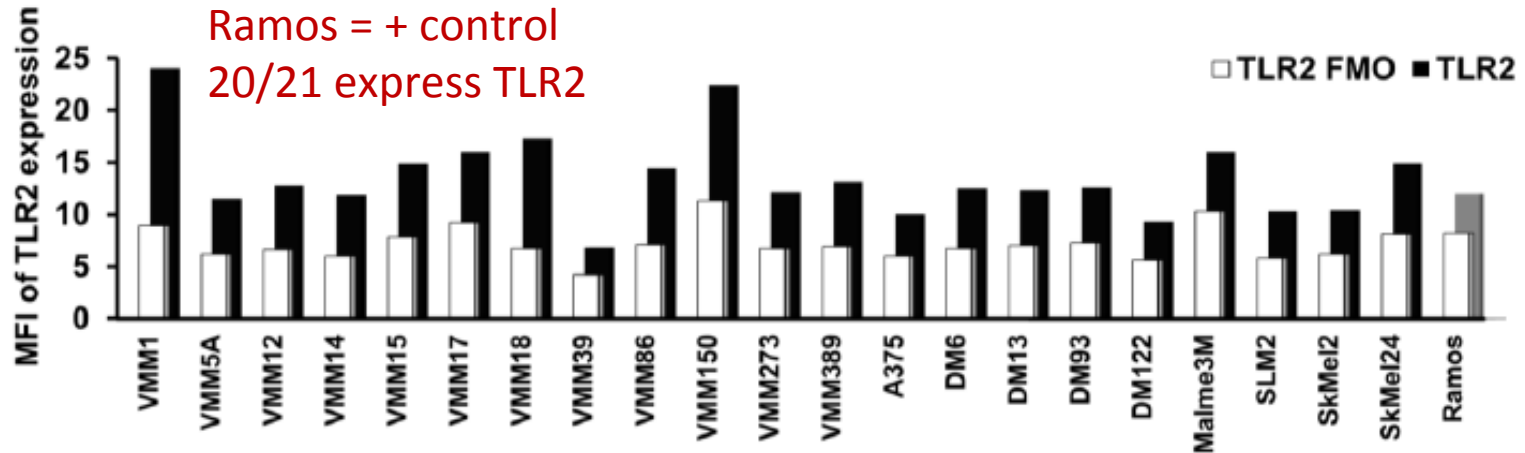
- TLR2/6 agonists outperform all others.
- 2 logs less MALP-2 needed than LPS to have a similar effect on CXCL10 production.



# TLR2 & TLR6 are expressed broadly on human melanoma cell lines.

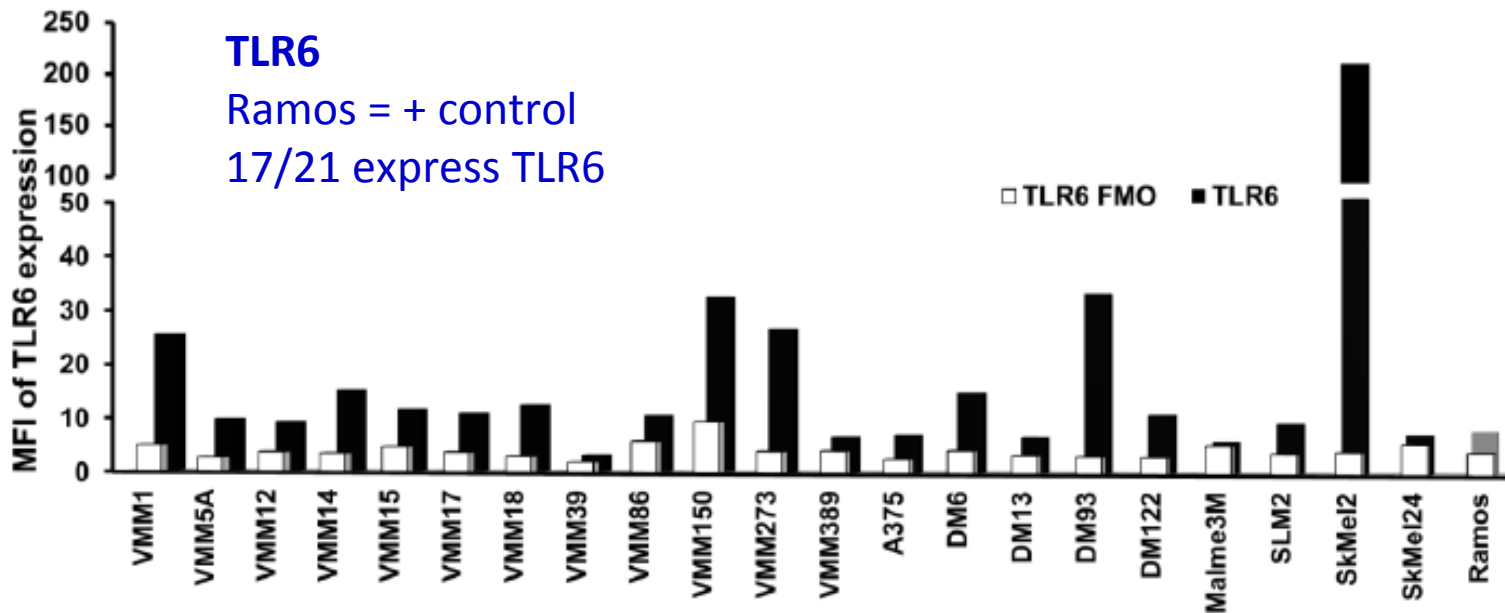
## TLR2

Ramos = + control  
20/21 express TLR2



## TLR6

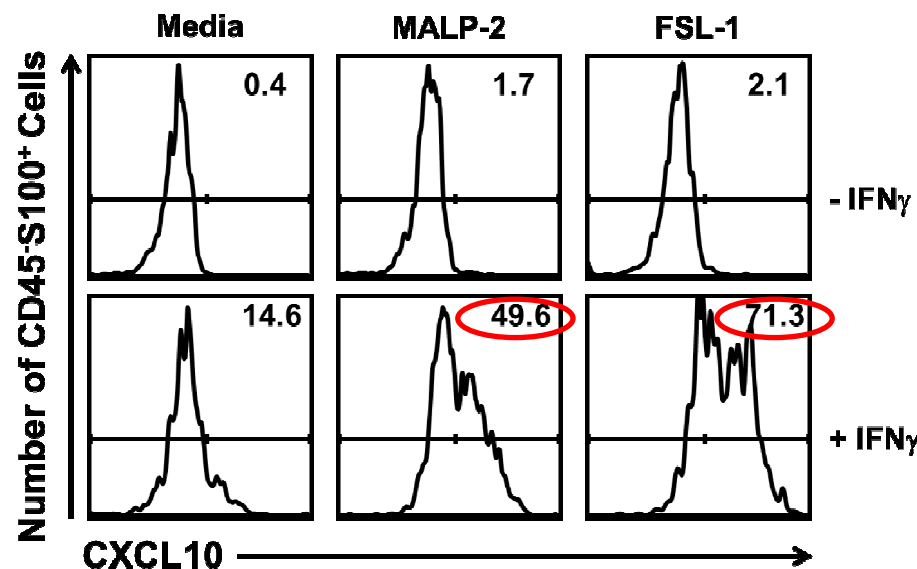
Ramos = + control  
17/21 express TLR6



# CXCL10 production is upregulated from freshly resected melanoma cells after stimulation with TLR2/6 agonists and IFN $\gamma$ , with patient- or site-specific variation

## Patient 1

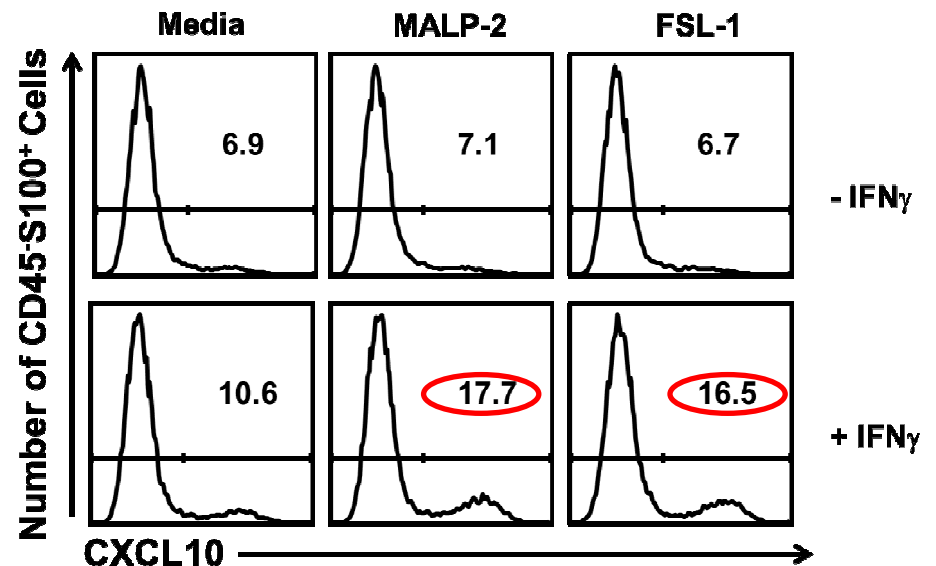
Melanoma CD45-S100<sup>+</sup>



Metastasis to R axillary node 9 years after surgery and vaccine trial for stage IIIA melanoma - **Immunotype A.**

## Patient 2

Melanoma CD45-S100<sup>+</sup>

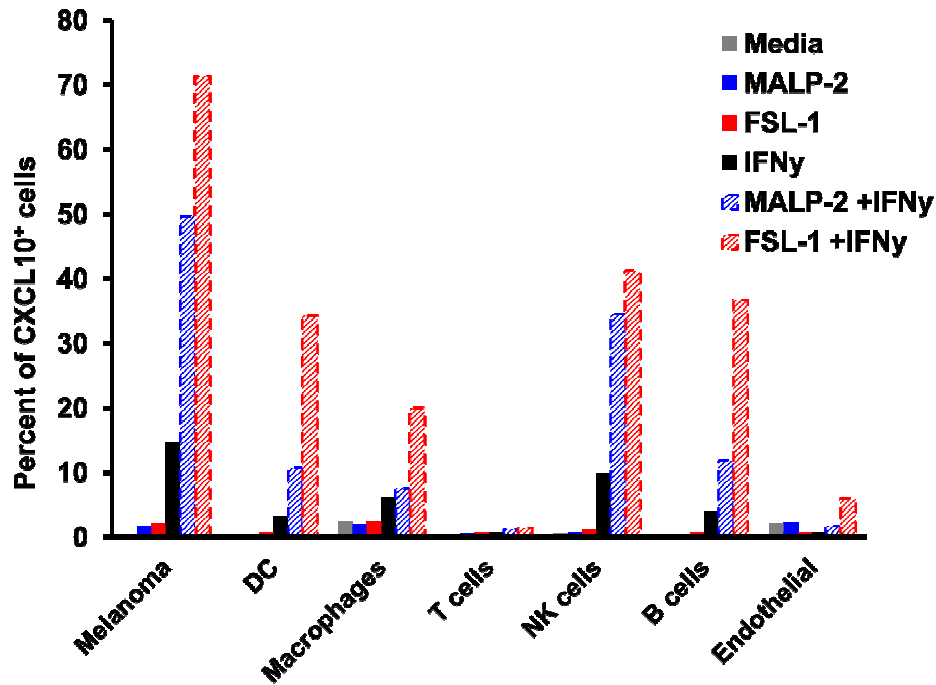


Metastasis to small intestine (with bleeding) 3 years after surgery and IL-2 for stage IV melanoma - **Immunotype C.**

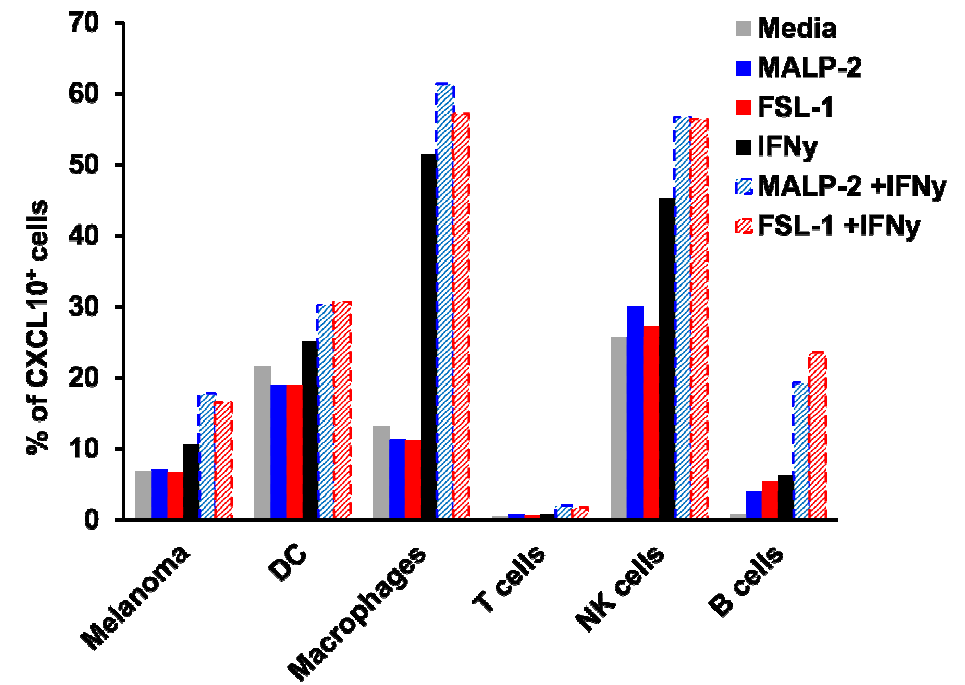


# CXCL10 production is upregulated from some immune cell types in patient tumors after stimulation with TLR2/6 agonists and IFN $\gamma$

Patient 1



Patient 2



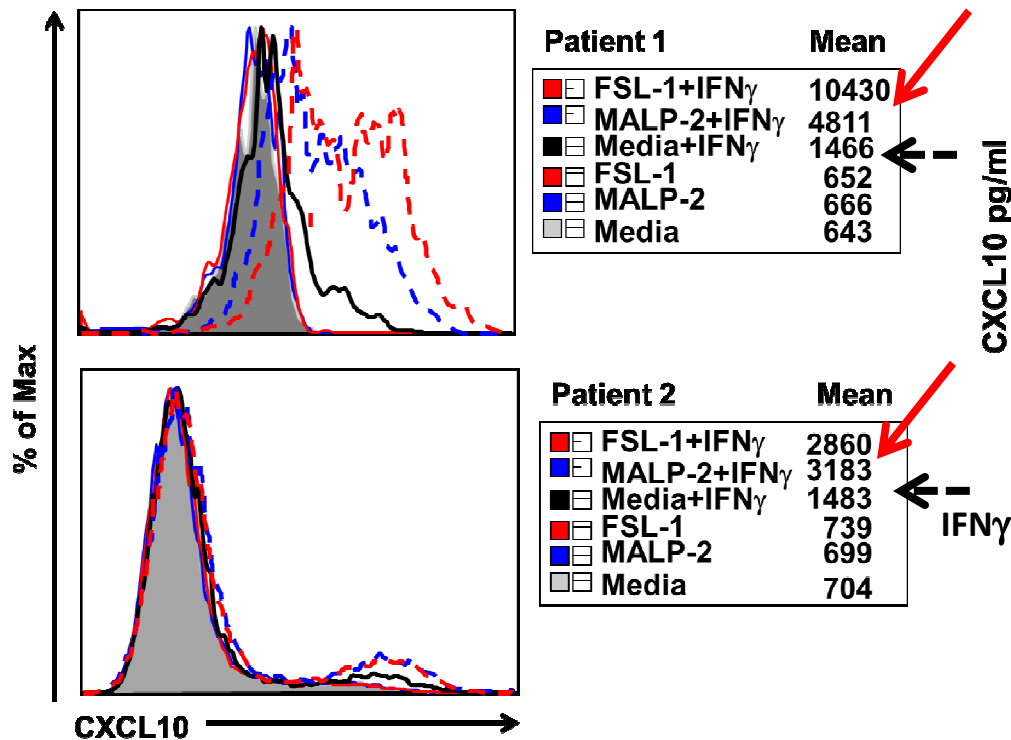
- Some constitutive production of CXCL10 by cells in patient 2 small bowel metastasis, may be due to bacterial presence of intraluminal ulcerated tumor.
- Not shown: CCL3 was not detected from freshly resected tumor specimens.



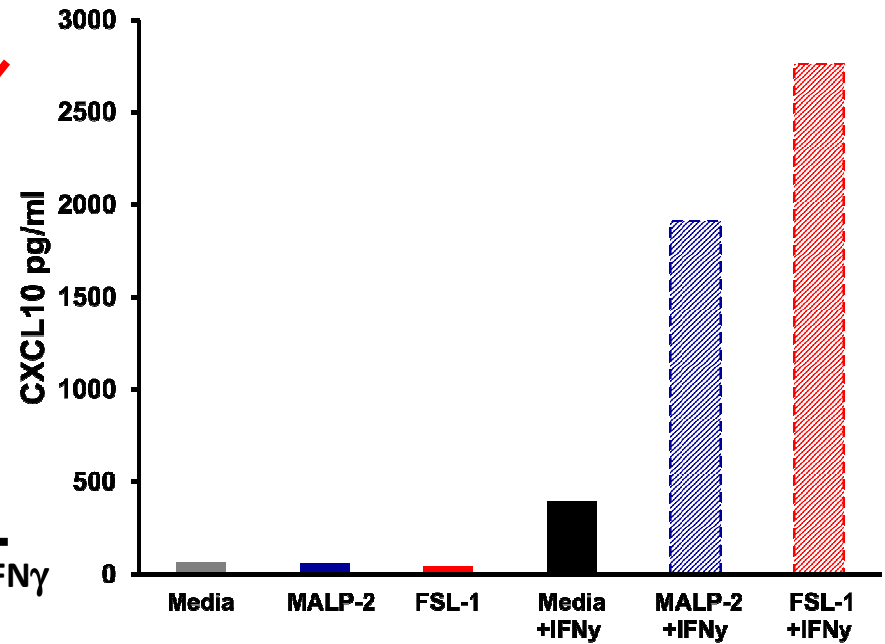
# CXCL10 production is upregulated from freshly resected melanoma cells after stimulation with TLR2/6 agonists and IFN $\gamma$

## MFI of CXCL10

Melanoma Cells



## ELISA assay for CXCL10 production



# Conclusions

- TLR2/6 agonist MALP-2 or FSL-1 and IFN $\gamma$  stimulation significantly improves CXCL10 production from melanoma cells.
- TLR2 and TLR6 are widely expressed on human melanoma cells.
- Freshly resected melanoma specimens also upregulate CXCL10 production with TLR2/6 agonists and IFN $\gamma$  stimulation observed from melanoma cells and immune cells subsets (DCs, macrophages), with some variability seen in the intensity of upregulation of CXCL10 observed.
- These data identify a novel synergy of TLR2/6 agonists and IFN $\gamma$  for inducing CXCL10 production directly from melanoma cells.
- Suggest that intralesional administration TLR2/6 agonists+IFN $\gamma$  may have value in combination with other immune therapies, by supporting T cell migration into melanoma tumors.*



The background of the slide is a photograph of a university campus. In the foreground, there are several trees with autumn-colored leaves in shades of orange, yellow, and red. In the middle ground, a large, white, classical-style building with a prominent dome and columns is visible. The sky is a clear, light blue with some wispy white clouds. The overall scene is bright and pleasant.

# *Thank You!*

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