Melanoma gene expression profiles to identify mechanisms of tumor resistance

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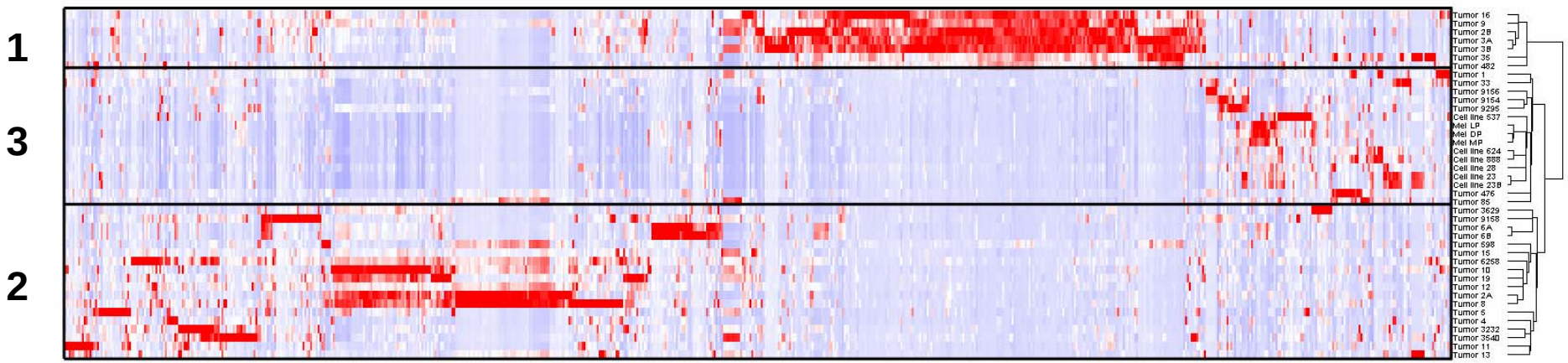
Introduction

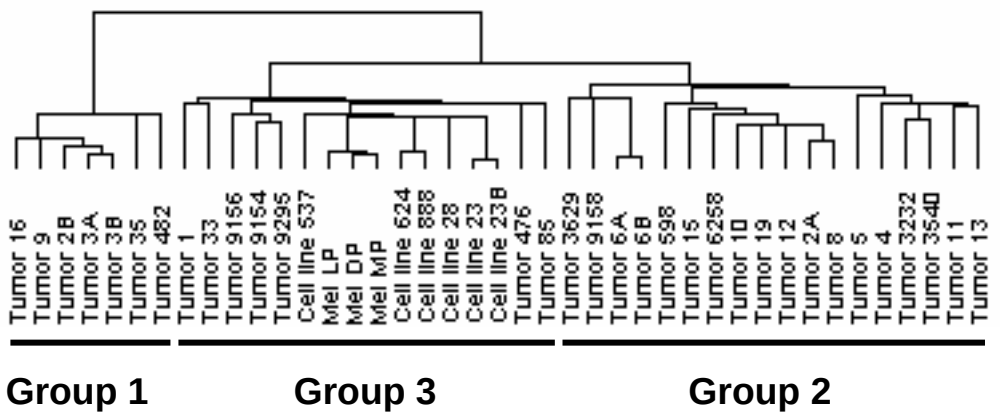
- High frequencies of melanoma antigen-specific $CD8^+$ T cells induced by vaccination or adoptive transfer do not always translate into tumor regression
- This observation has prompted investigation into the tumor microenvironment to identify potential factors that positively or negatively regulate the effector phase of an anti-tumor immune response
- One approach has been to characterize gene expression profiles from metastatic melanoma tumors (core biopsies or resected specimens)

Methods

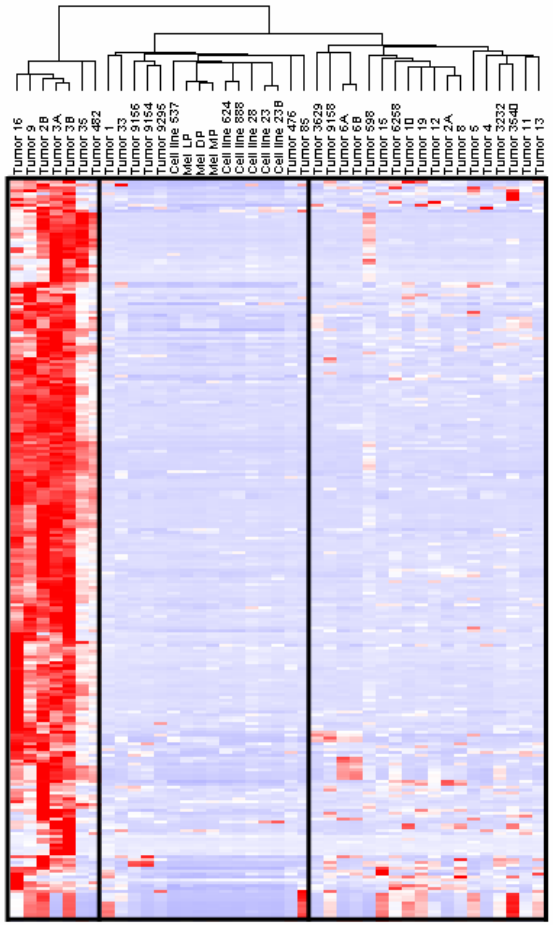
- RNA was extracted from tumor biopsies of patients with metastatic melanoma (n=33) as well as from melanoma cell lines (n=5) and primary melanocyte cell lines (n=3)
- Gene array analysis was performed (Affymetrix chip U133A)
- Data was analyzed using dChip software, filtering for genes present in >10% of samples and with variation of std. dev./mean between 1 and 10. 735 genes with variable gene expression were identified.
- Genes and samples were clustered using hierarchical cluster analysis.
- In some cases, gene expression was analyzed using real time RT-PCR, both to confirm gene array data and to look for expression of genes not detected by the chip.

The gene array samples cluster into 3 main groups





Tumor group 1 expresses immunologically relevant transcripts



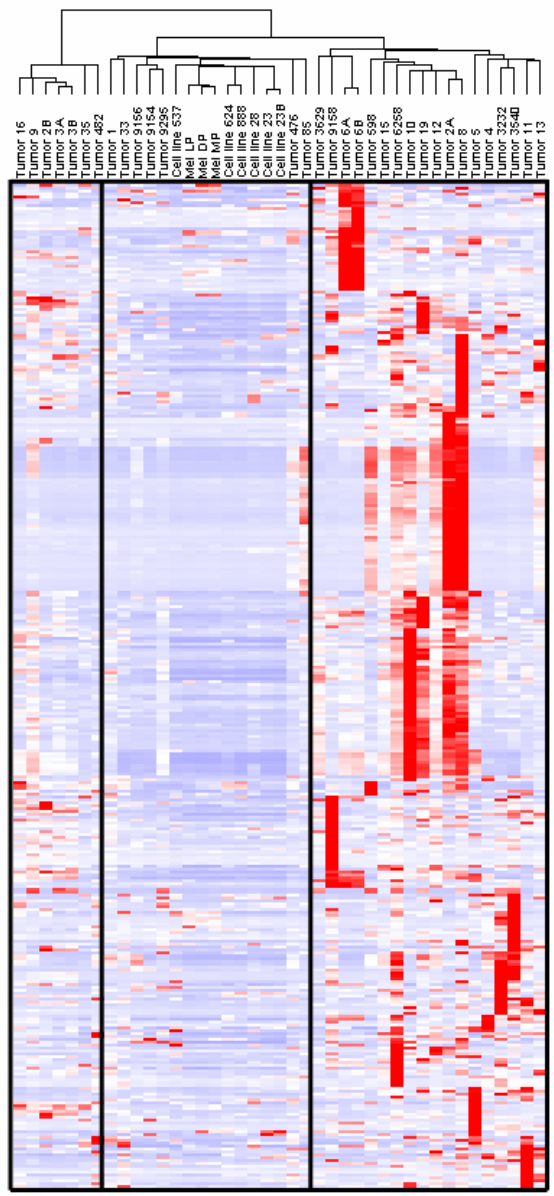
Putative positive factors:

- **Cytokines (IL-18)**
- **Chemokine (CCL27)**
- **Cytokine receptor (IL-1R)**

Putative negative factors:

- **Arginase**
- **IL-1R antagonist**

Tumor group 2 expresses T cell, B cell, and other immune cell transcripts



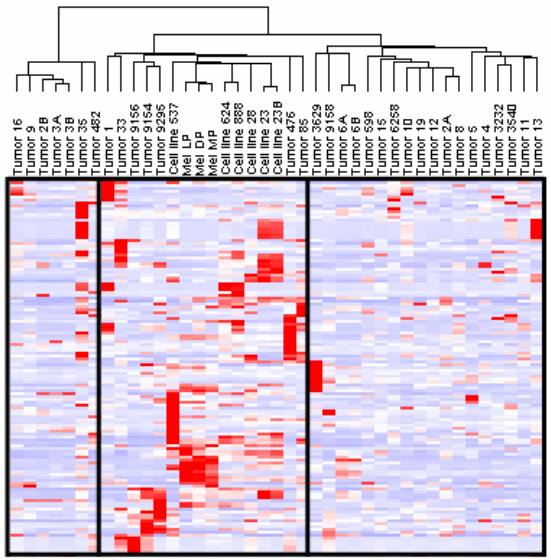
Putative positive factors:

- **B cell markers (IgH, Igκ, Igλ)**
- **T cell markers (TcRα, β, γ)**
- **MΦ markers (MΦ scavenger receptor 1)**
- **Complement components**
- **Granzyme A, K**
- **MHC class II**
- **Chemokines (CXCL13, CXCL10, CXCL11, CXCL9, CXCL5, CCL5)**

Putative negative factors:

- **Indoleamine-pyrrole 2,3 dioxygenase (IDO)**
- **Tryptophan 2,3-dioxygenase**
- **Angiogenesis factors (angiopoietin 2, angiopoietin 1, VEGF)**

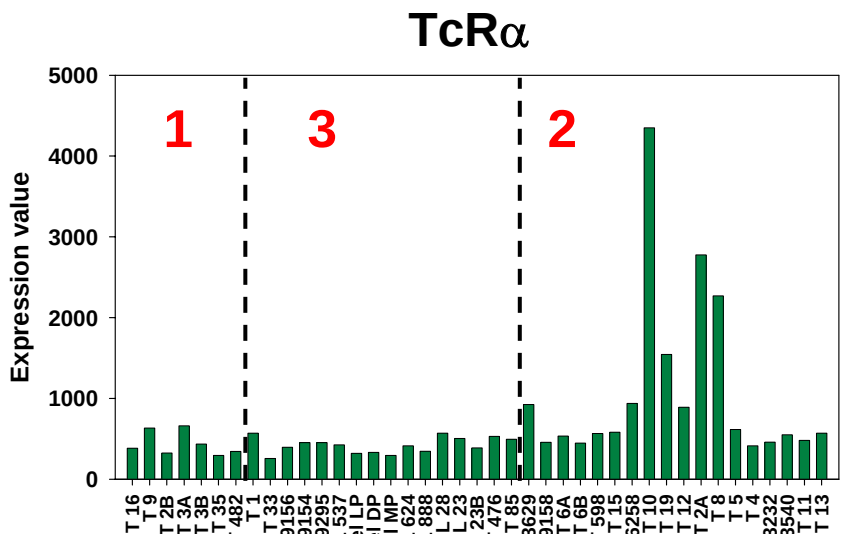
Tumor group 3 is characterized by a lack of immunologically relevant transcripts and includes melanoma and melanocyte cell lines

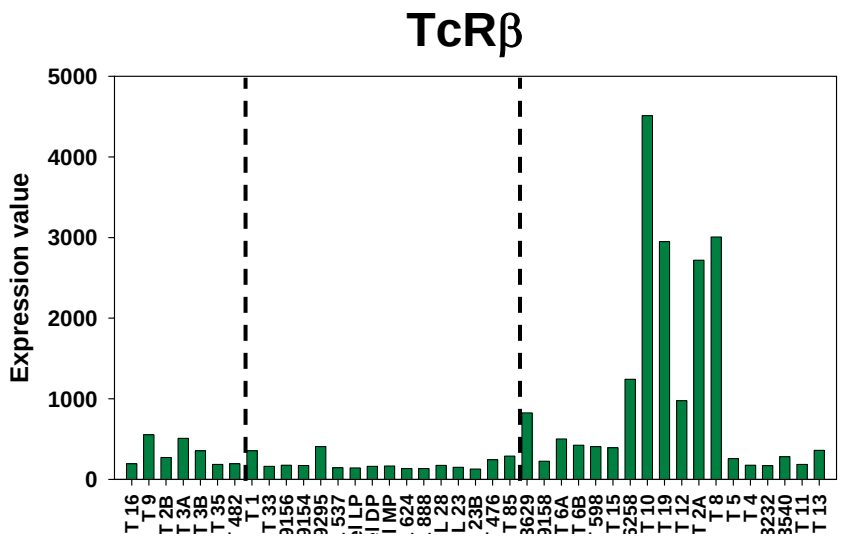


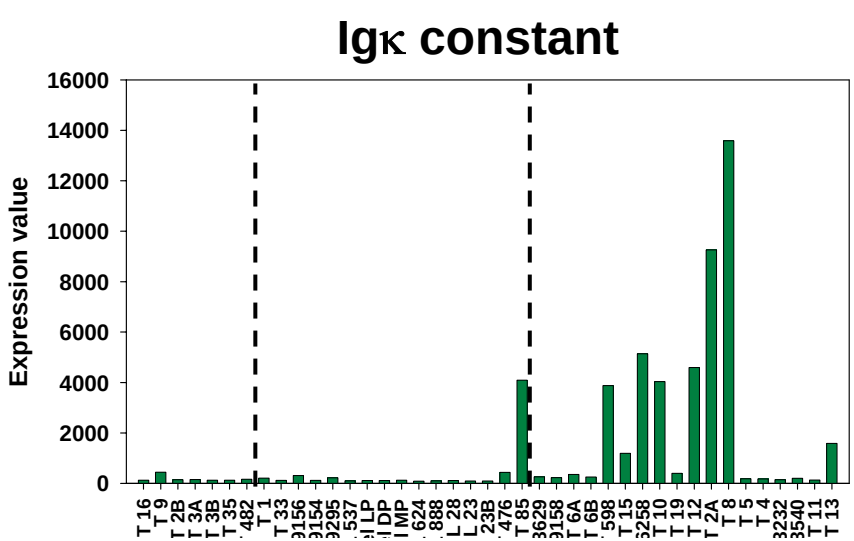
Expression of various tumor markers:

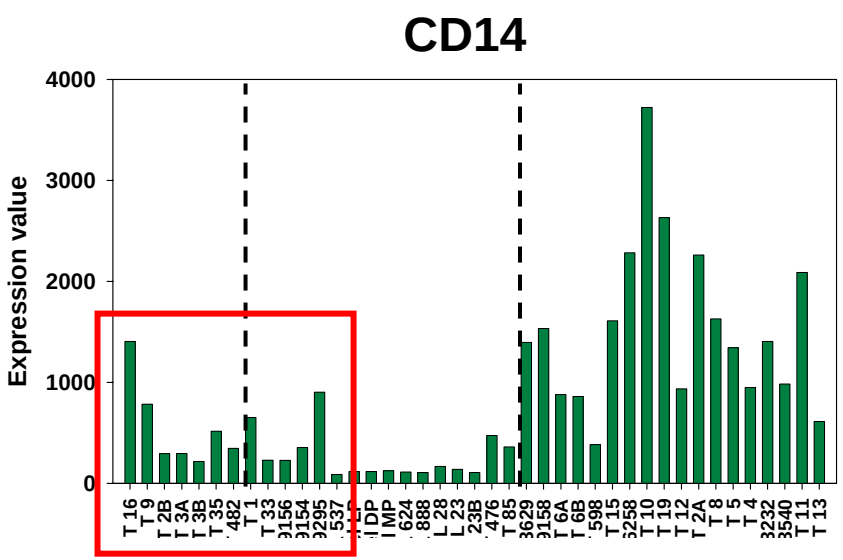
- **Cancer/testis Ags**
- **Melanoma differentiation Ags**
- **Oncogenes**

T cell, B cell and Mϕ transcripts are expressed highly in group 2 tumor samples

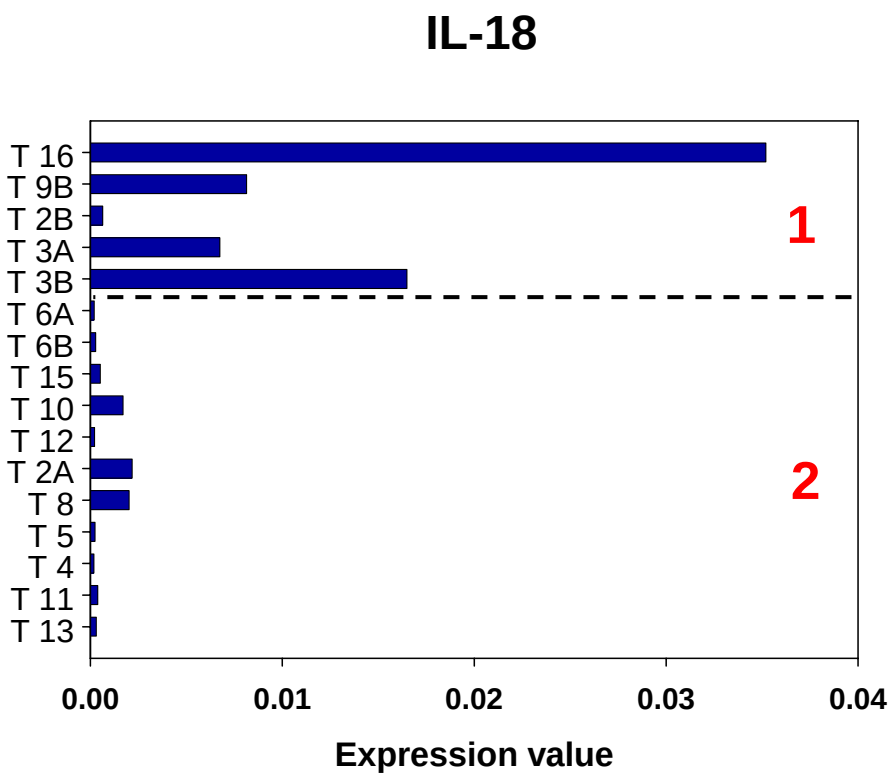


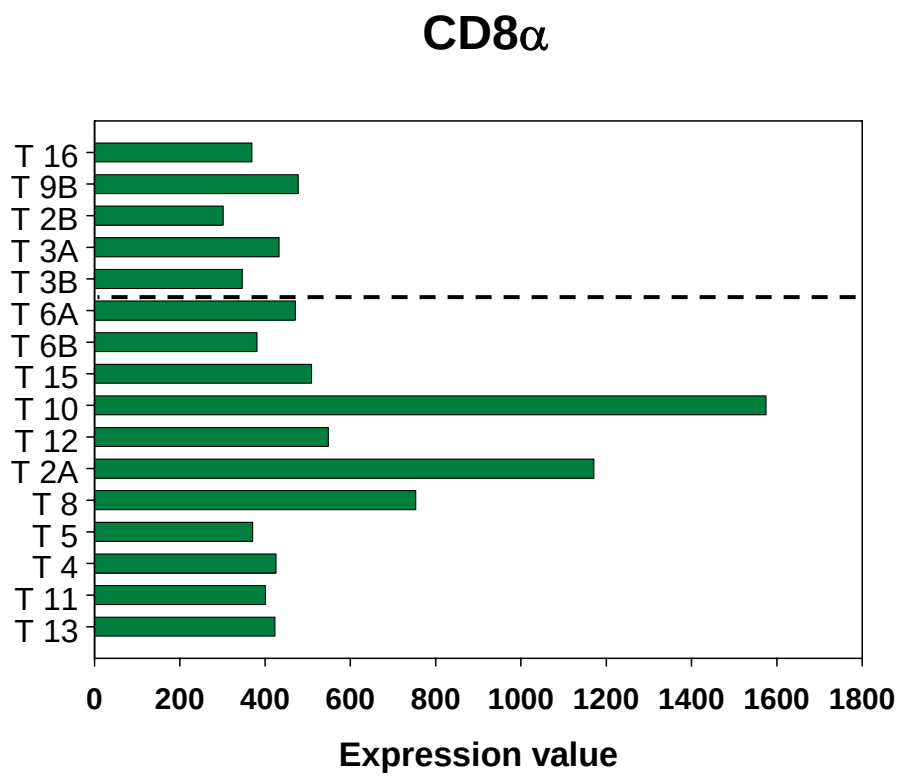




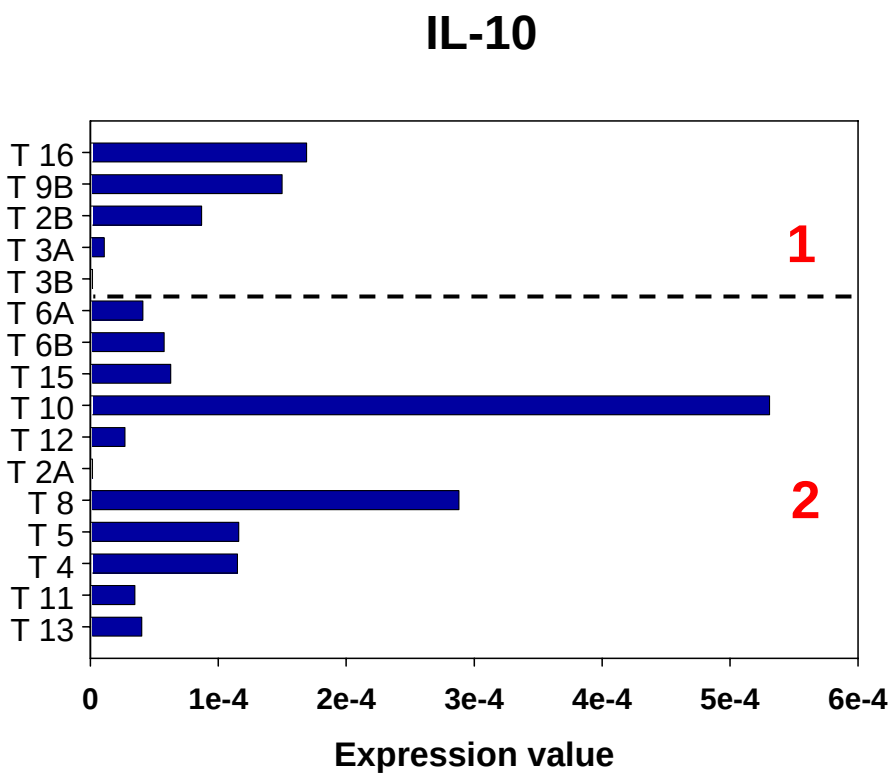


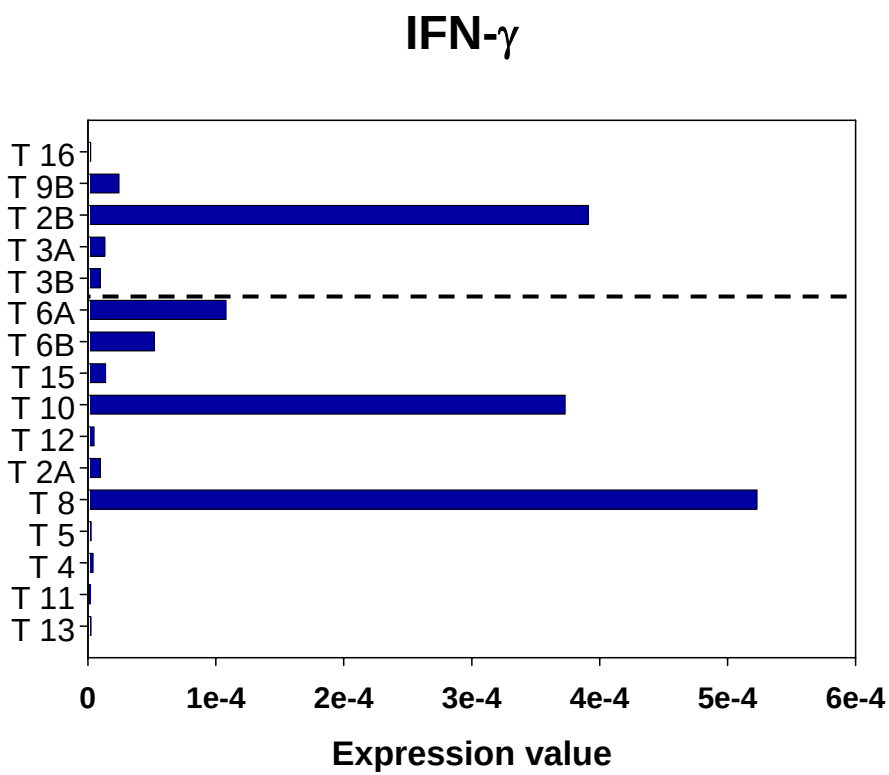
Representative transcripts encoding “positive” immune factors in tumor groups 1 and 2



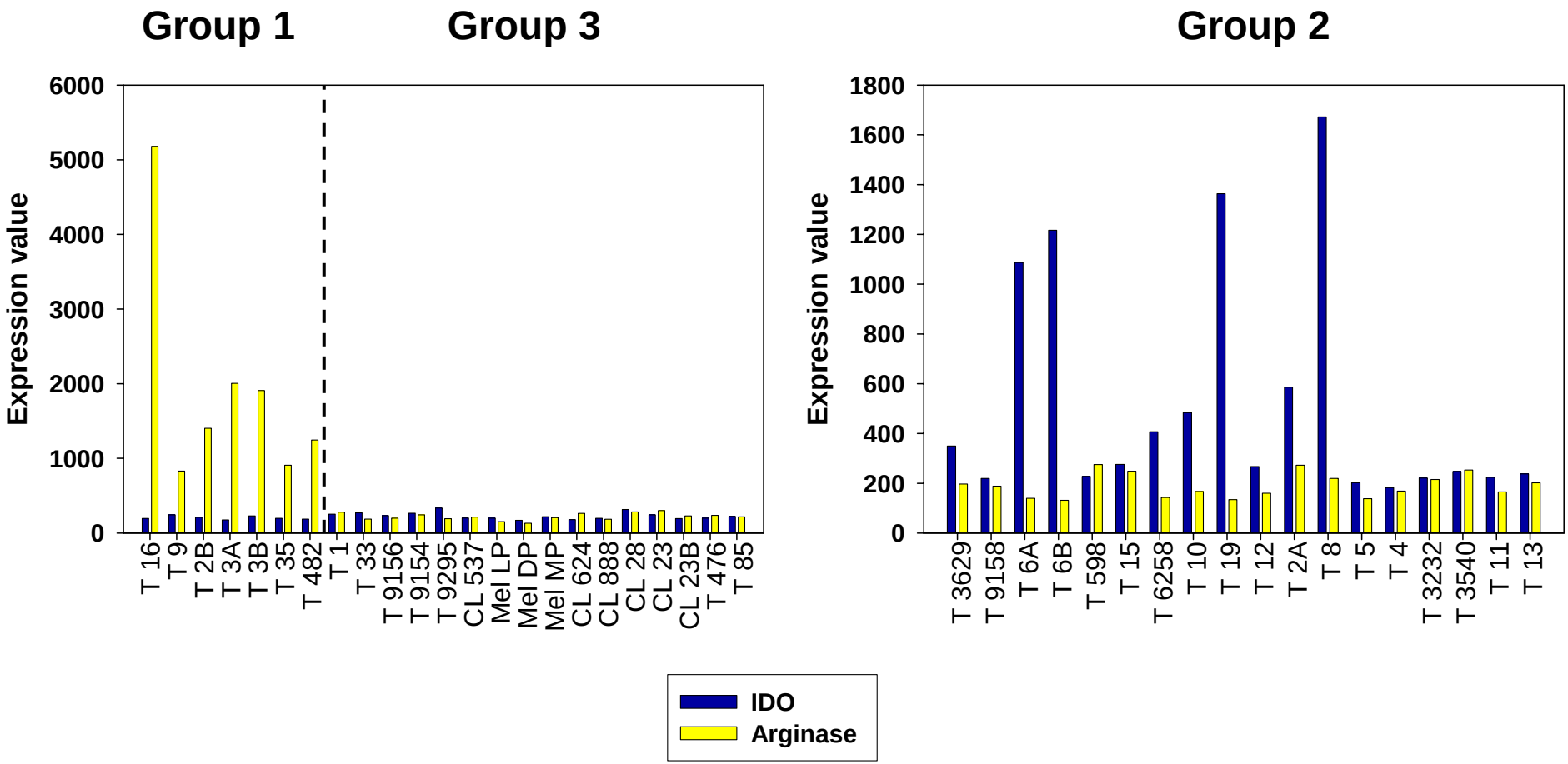


IL-10 and IFN-γ transcripts are present variably in tumor samples from both group 1 and group 2

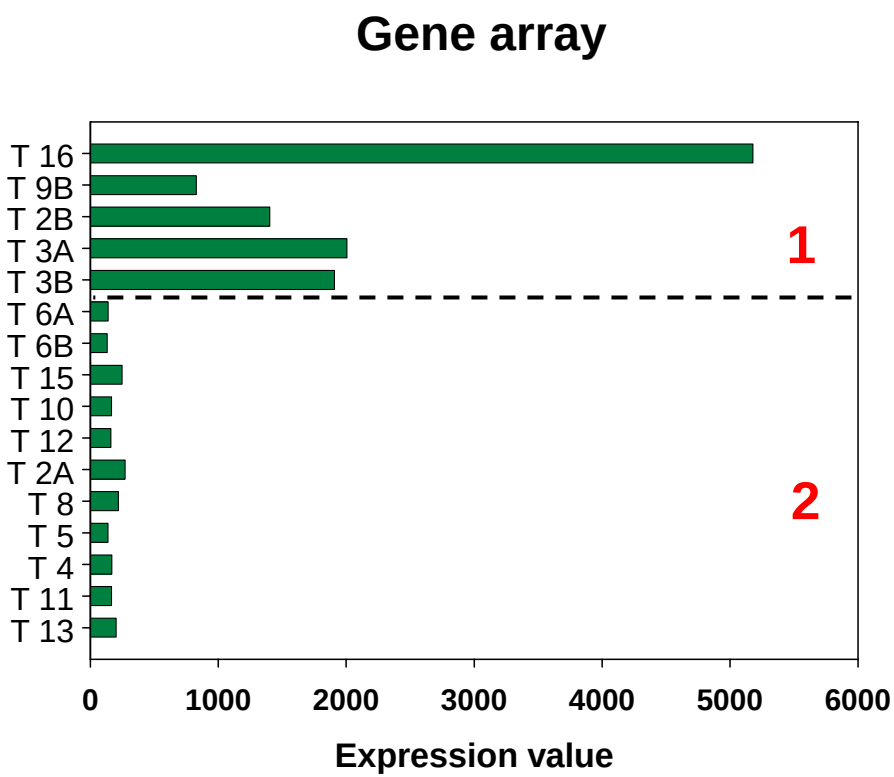


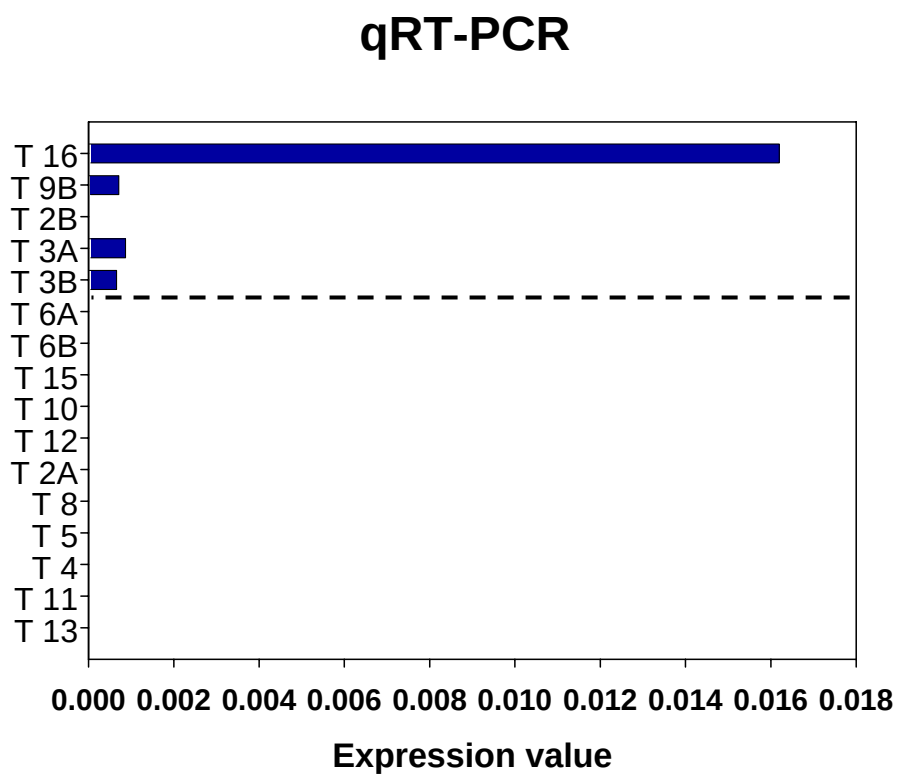


Arginase and IDO expression levels show an inverse correlation

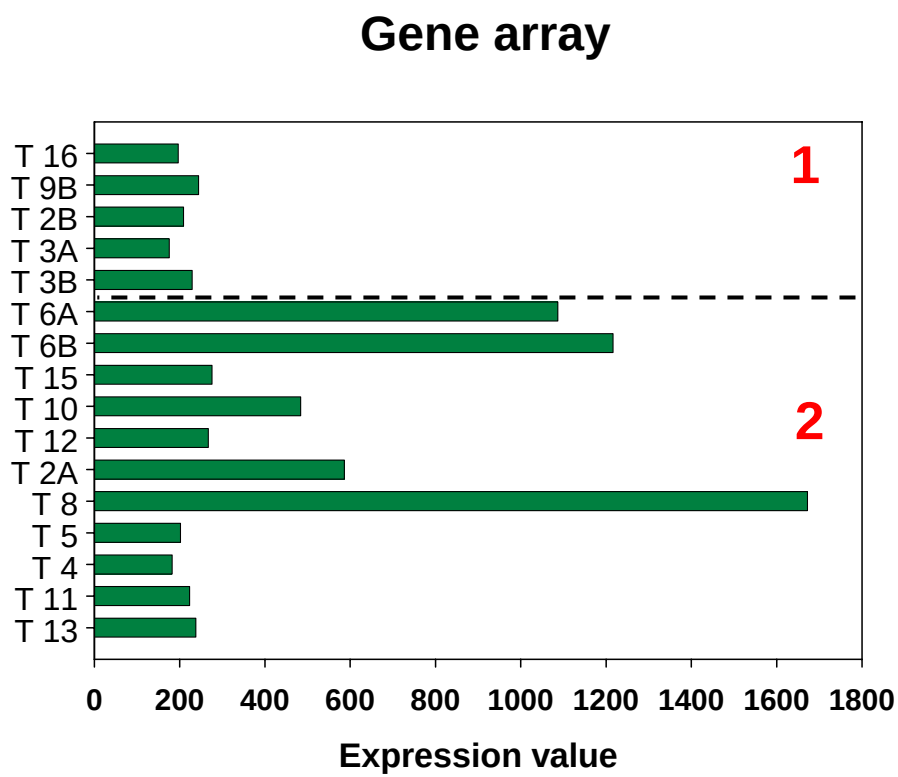


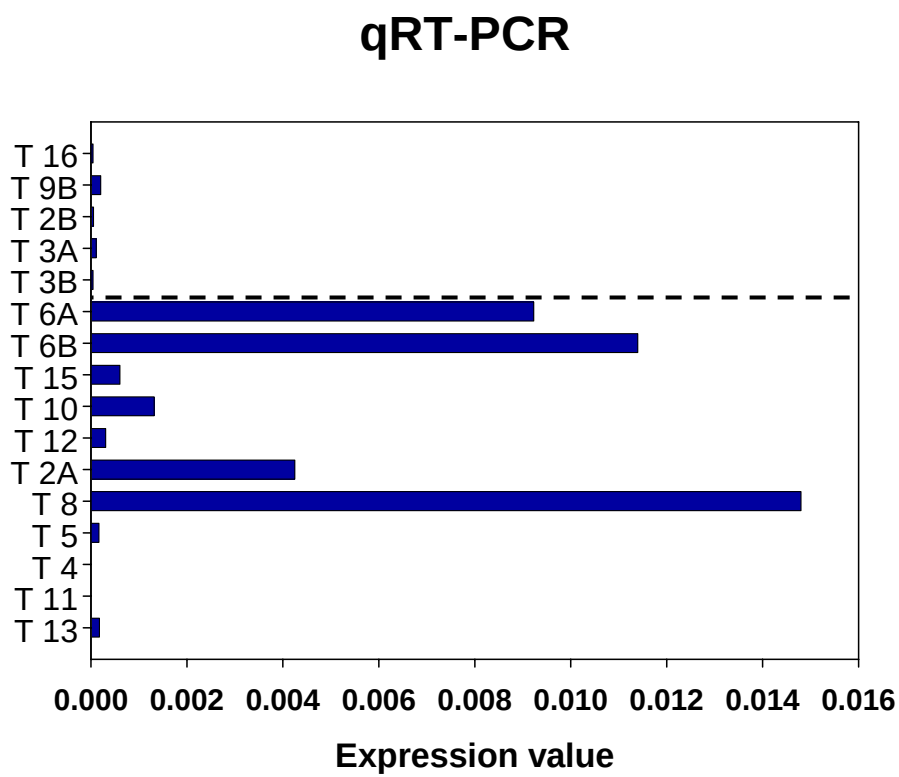
Arginase expression validated by real-time RT-PCR is primarily found in group 1 samples



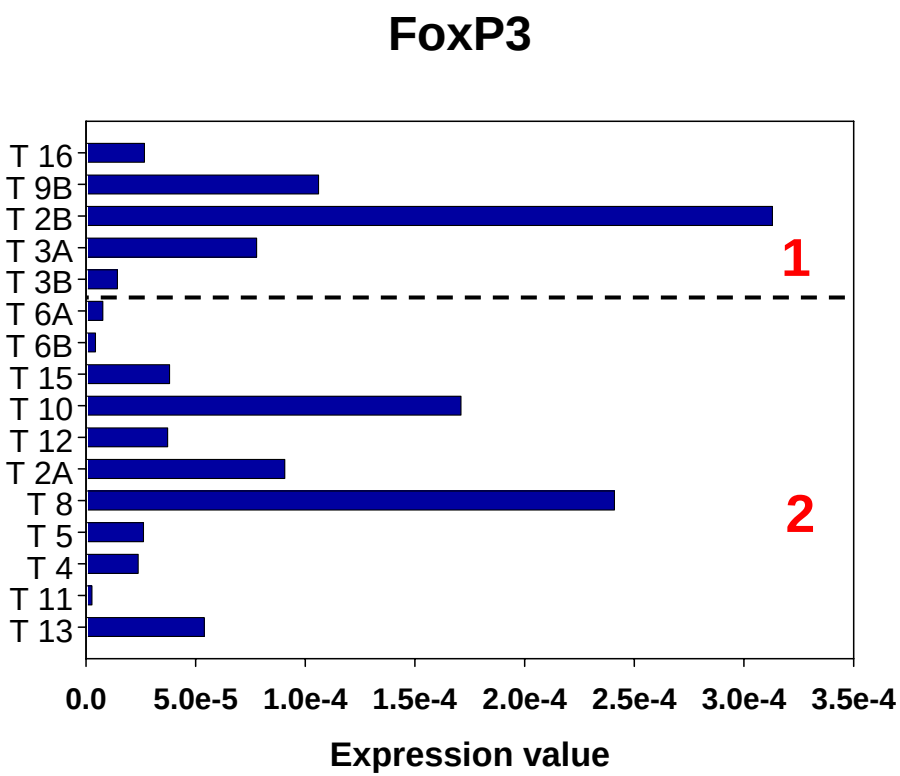


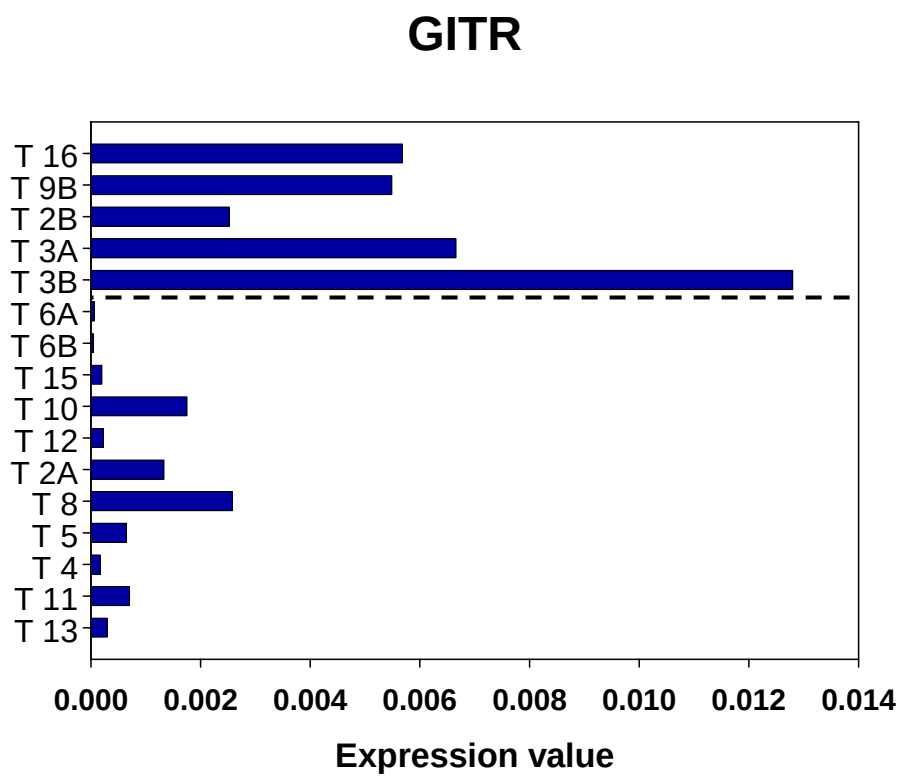
IDO expression validated by real-time RT-PCR is primarily found in group 2 samples



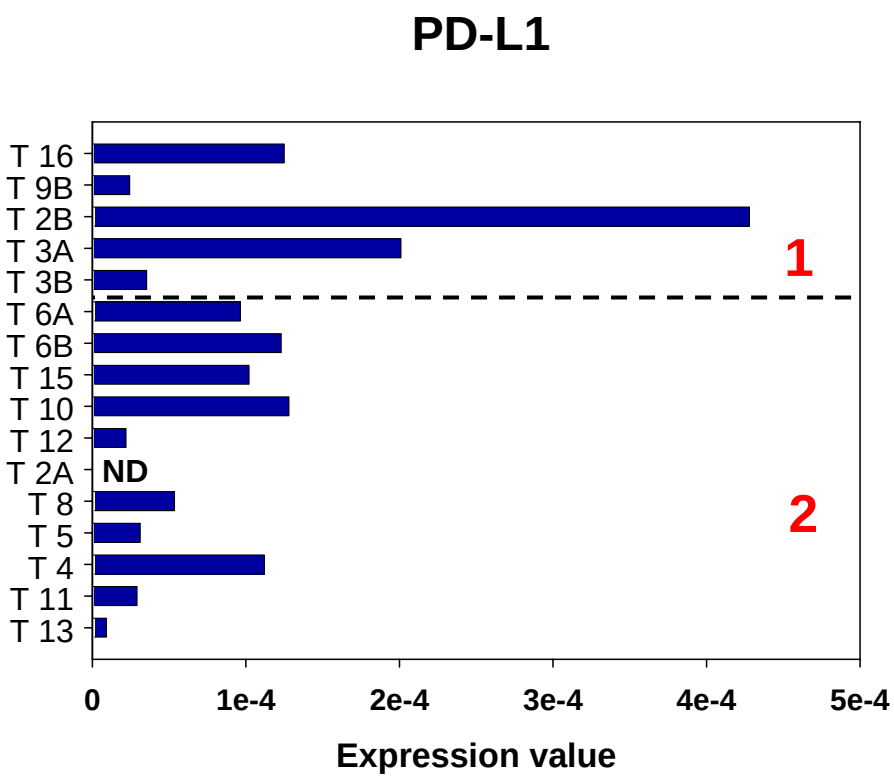


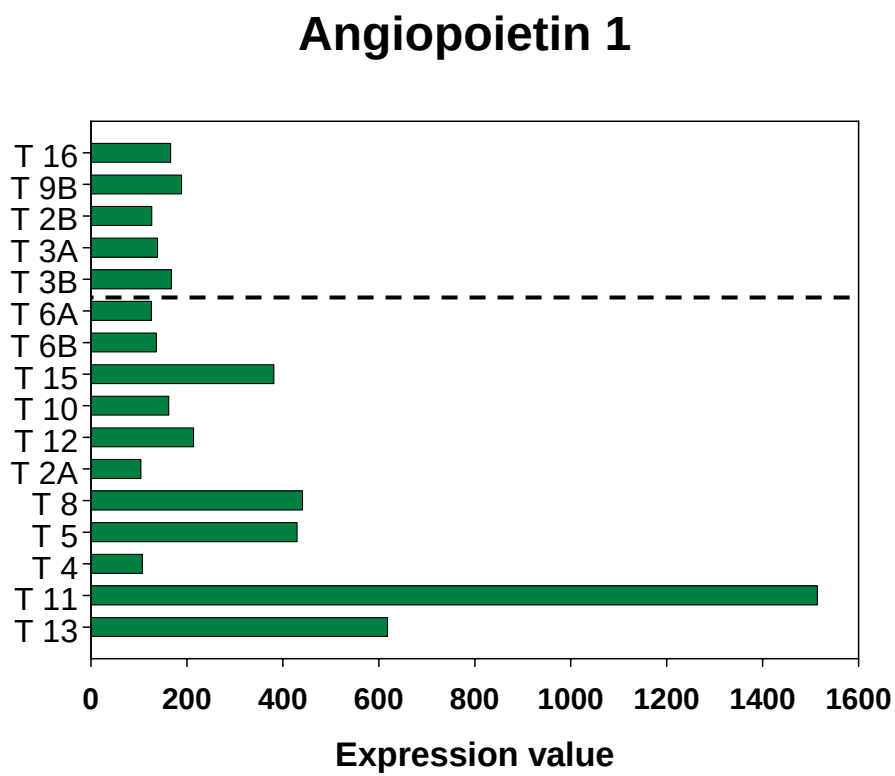
Regulatory T cell transcripts in tumor groups 1 and 2



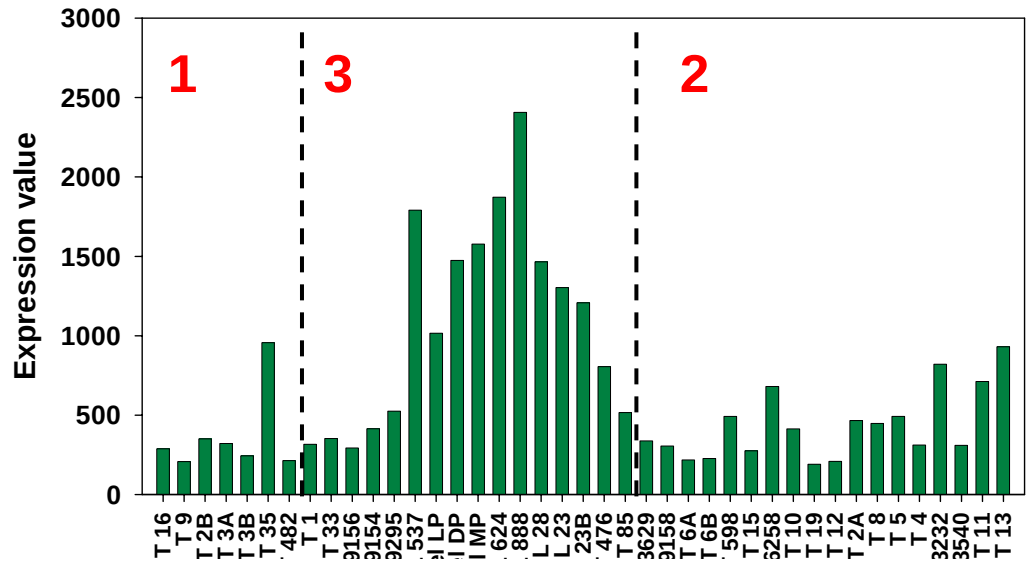


Additional transcripts encoding putative negative regulators





Survivin transcripts are widely expressed, being highest in group 3 tumor cell line samples



Conclusions

- Metastatic melanoma tumor samples cluster into at least three groups based on gene expression profiling
- Groups 1 and 2 have distinct gene expression patterns
  - Both contain distinct sets of immunologically relevant genes
  - Include putative negative and positive regulators of T cell function
- Group 3 lacks immune genes and clusters together with melanoma and primary melanocyte cell lines
  - Suggests defects in inflammatory cell trafficking
- Group 1 samples express Arginase and group 2 contains all samples with IDO. Arginase and IDO expression are inversely correlated.
- Therefore, all tumors appear to be accounted for by a putative negative immunoregulatory process:
  - Lack of inflammatory cell migration
  - Expression of inhibitory factors
- A larger number of samples is currently being analyzed, with planned correlation with clinical outcome
- Therapeutic strategies to counter inhibitory influences in the tumor microenvironment should be considered for development

Acknowledgments

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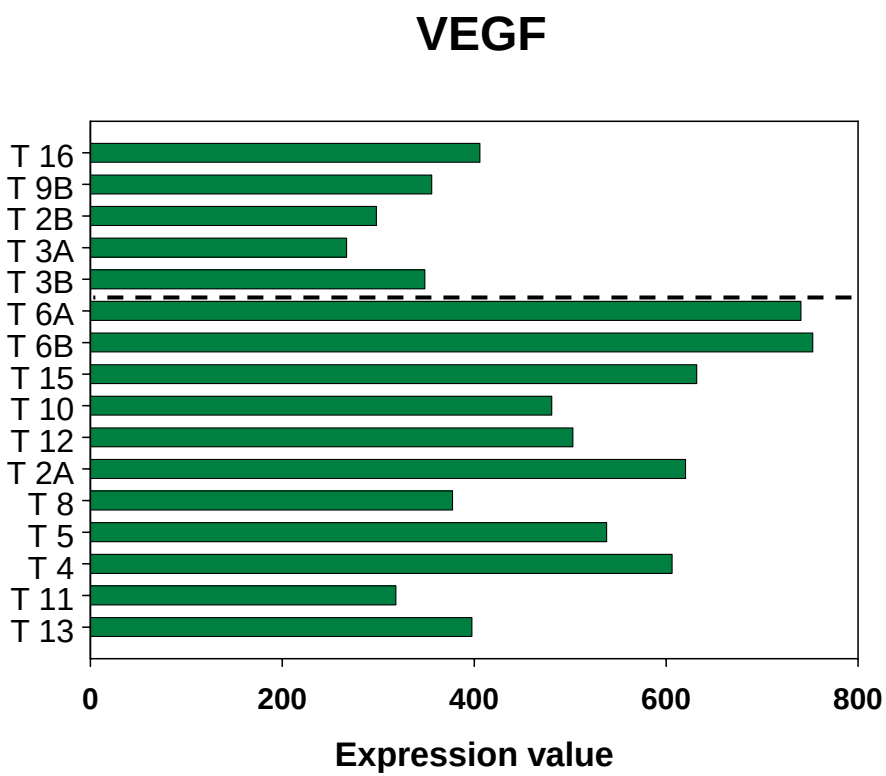
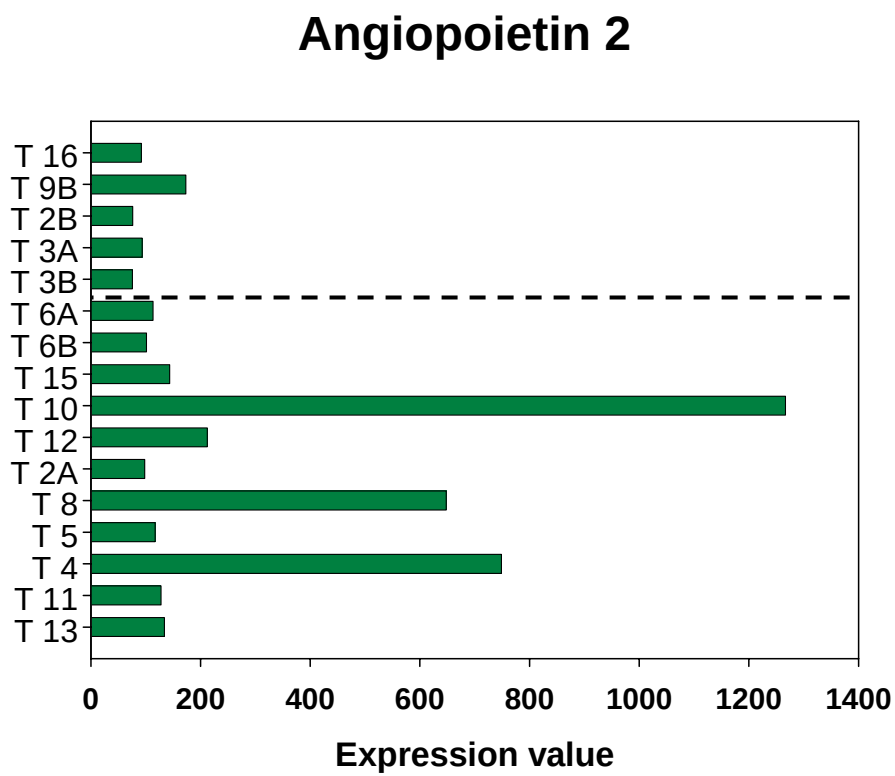
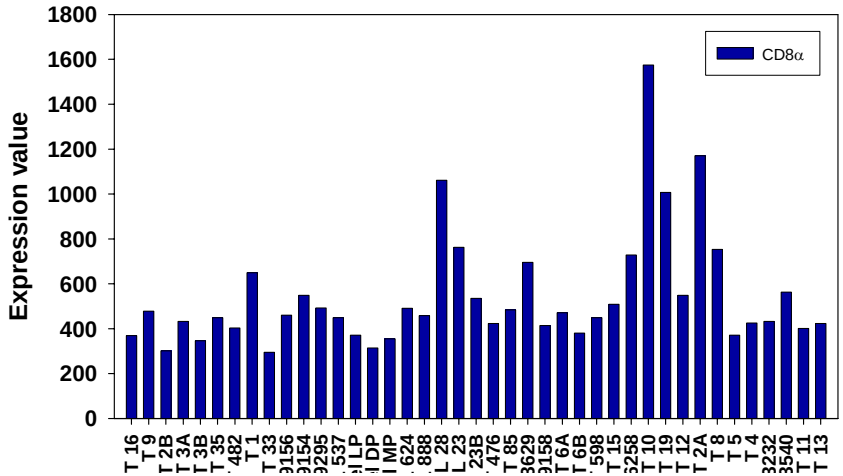
Angiogenic factors are primarily present among group 2 tumor samples

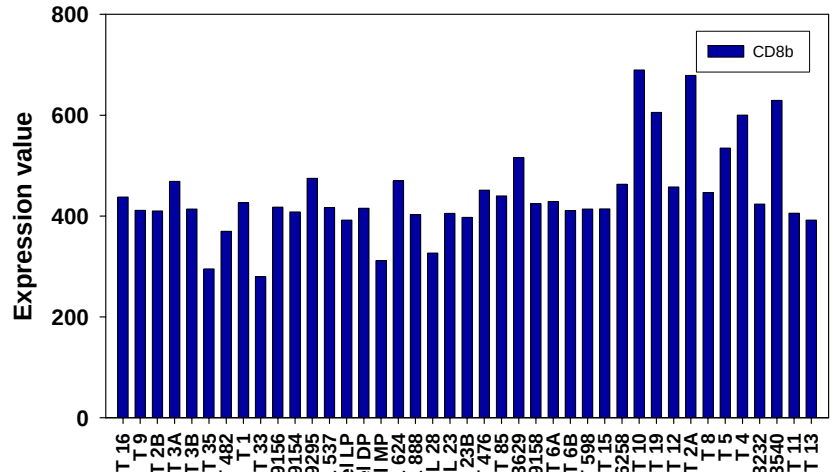




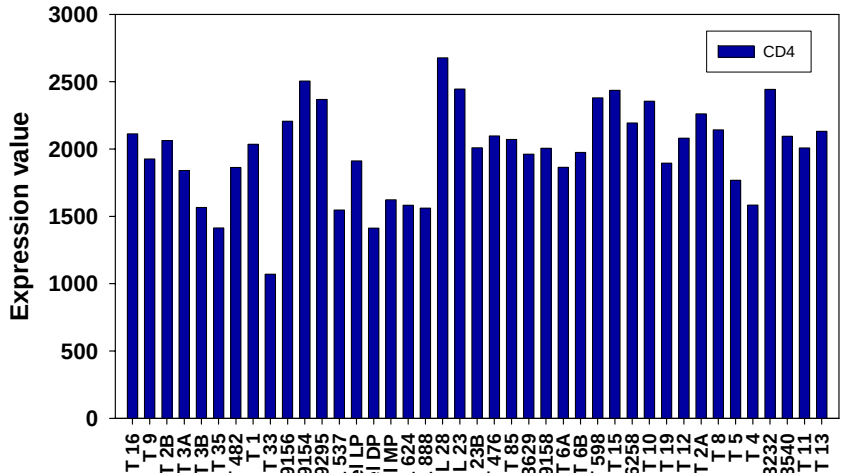
CD8α



CD8β



CD4



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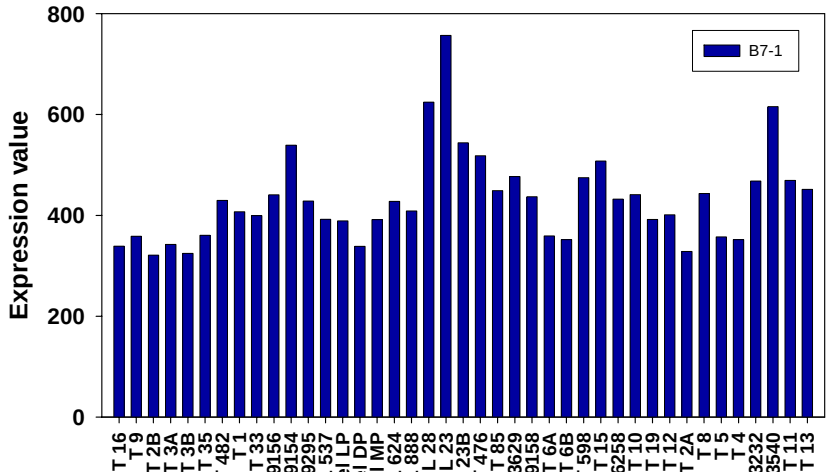

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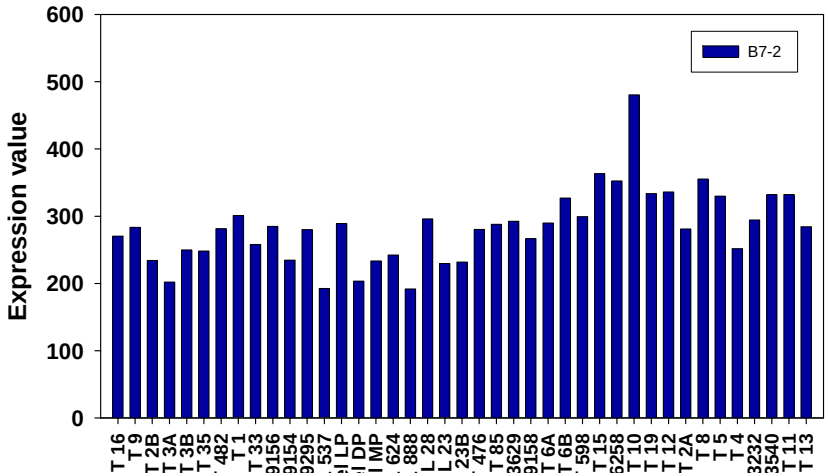
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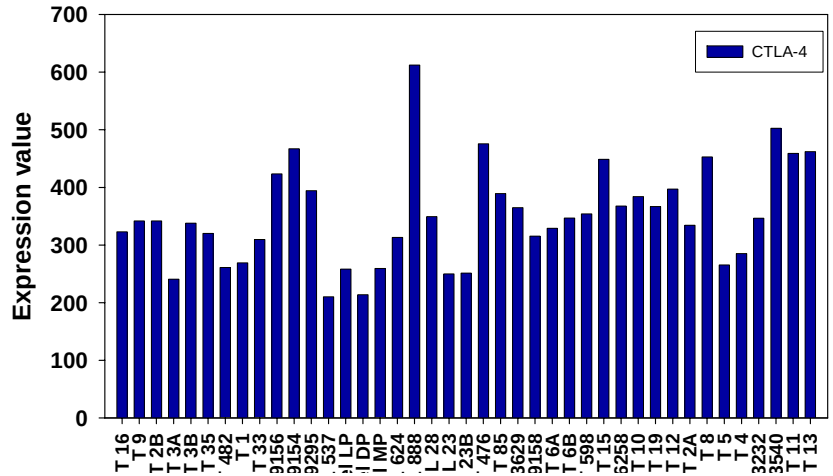
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