Presenter Disclosure Information

Michael P. Gustafson, Ph.D.

The following relationships exist related to this presentation:

No relationships to disclose.

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LOSS OF HLA-DR EXPRESSION ON CD14+ CELLS; A COMMON MARKER OF IMMUNOSUPPRESSION IN CANCER PATIENTS

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2010 iSBTC Annual Meeting

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Assessing patient immunity by peripheral blood immunophenotyping

- Flow Cytometry
- Whole blood
- Broad unbiased approach
  > 40 phenotypes
- Patients: prior to therapy or
  > 8 weeks off treatment

Diseases

Glioma Stage IV (GBM) n=50
Prostate Cancer n= 40
Non-Hodgkin's Lymphoma (NHL) n=40
Clear Cell Renal Cell Carcinoma (RCC) n=23
Chronic Lymphocytic Leukemia (CLL) n=29
Patients at risk for sepsis n=29
Diabetes and ALS n=11
TOTAL=193 patients

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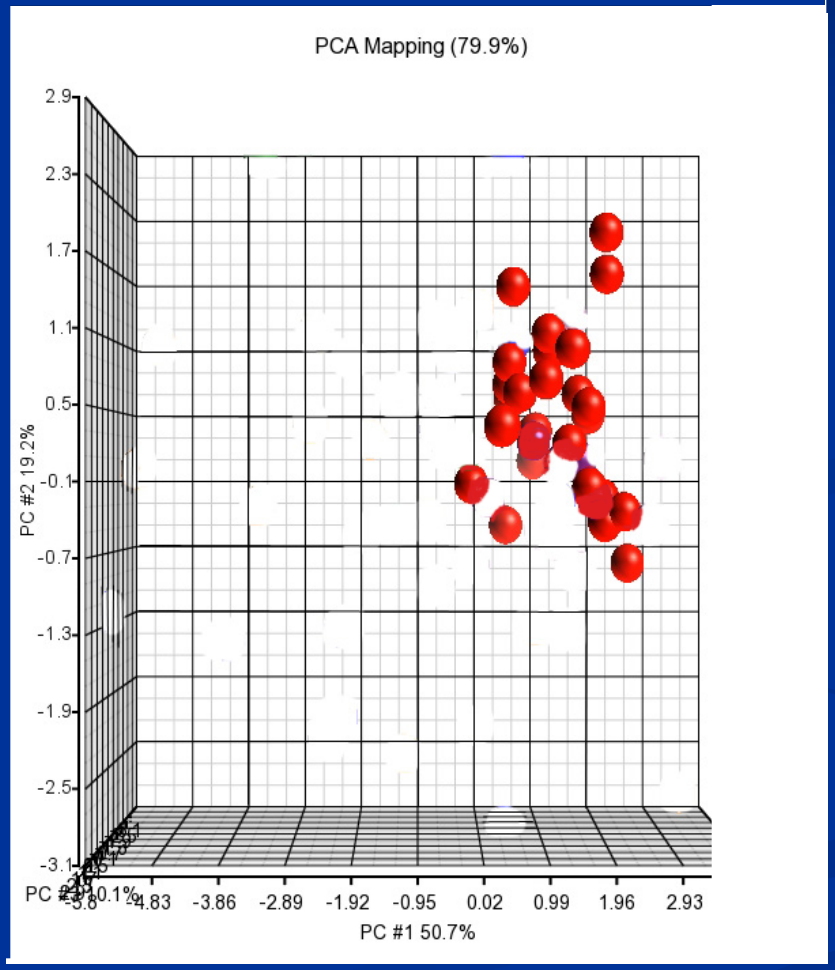
Lymphocytes
- **T cells**
  - **T helper/T killer subsets**
  - **T regulatory cells**
  - **Costimulatory/inhibitor receptors**
  - **Activated T cells**
  - **Central and effector memory**
  - **Naïve T cells**
- **B cells**
  - **Naïve and Mature B cells**
  - **Transitional B cells**
  - **Plasma cells**
  - **Isotyped switched**
- **NK cells**
  - **NKT cells**

Myeloid cells
- **Monocytes**
  - **M1 and M2 (CD14+ vs CD16+)**
  - **HLA-DR**
  - **Co-stimulatory receptors**
  - **Signaling receptors**
- **Granulocytes**
  - **CD15+CD66b+**
- **Myeloid Derived Suppressor cells**
  - **Classical Lin-HLA-DR-CD33+**
  - **"Granulocytic" CD15+CD14-**

Generating "Immune Profiles" using bioinformatics



- Phenotypes converted to cells/uL
- Data analyzed using Partek
- Each sphere representing individual immune profiles
- Clustering identifies relationships
- Provides 'unbiased' method to look for phenotypes of interest

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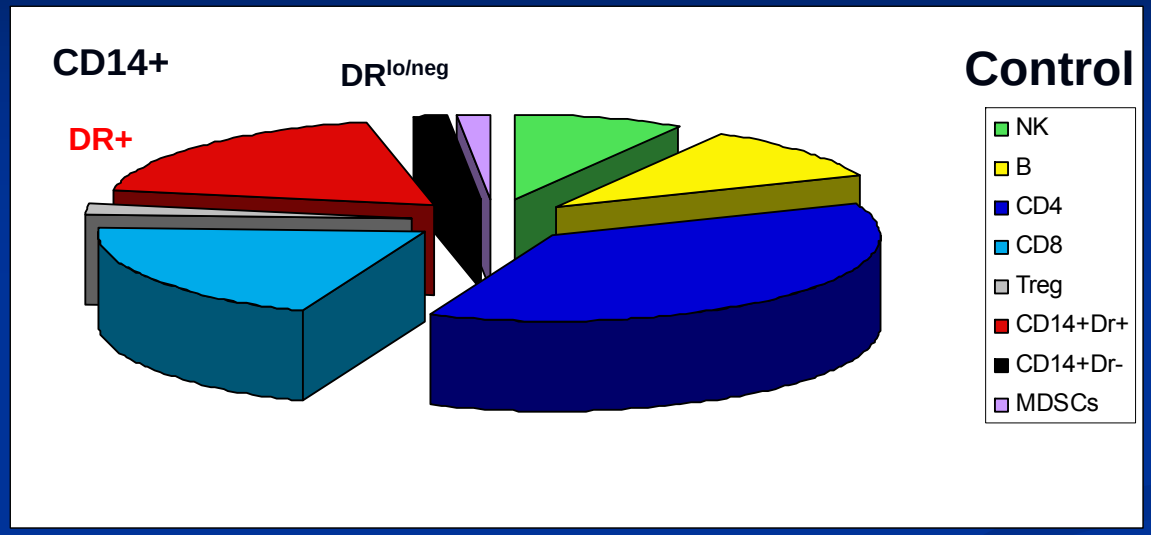
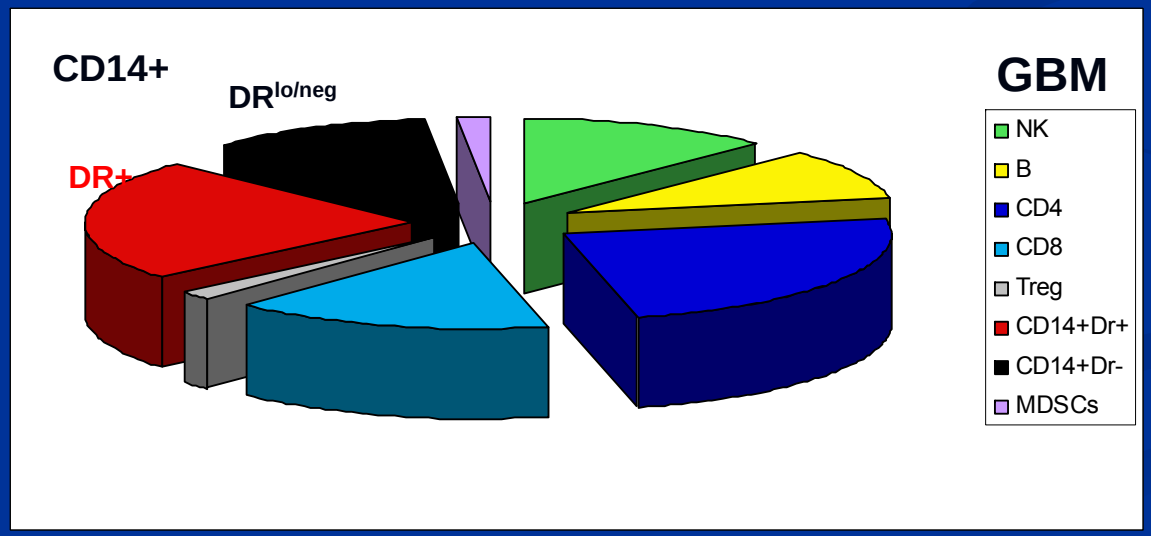
$CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes profoundly change the immune profile of patient PBMCs





With enough disease profiles, average profile of patients can be determined

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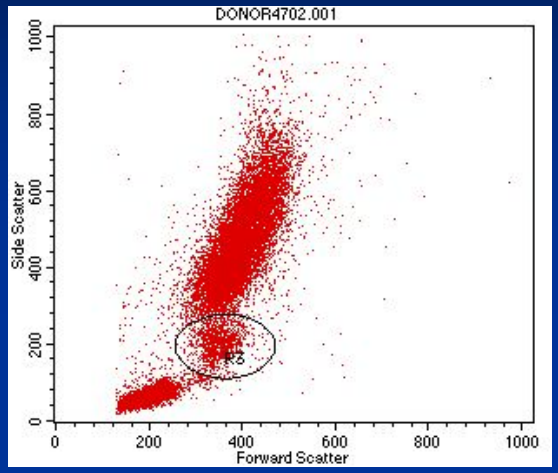
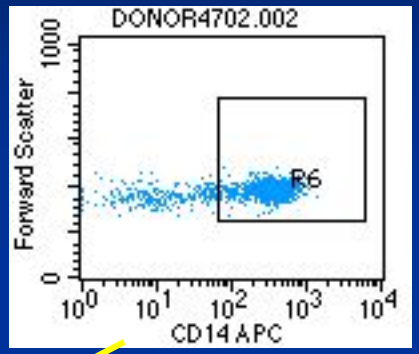
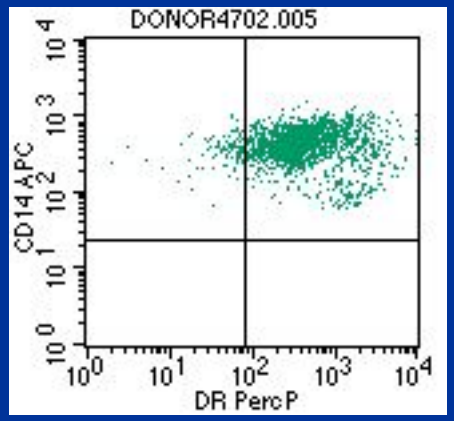
$CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes are elevated in cancer patients

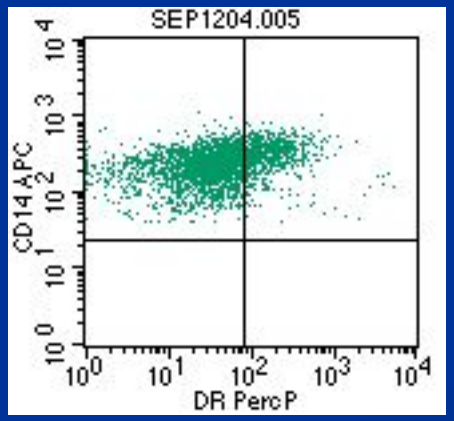




Normal Donor



Patient



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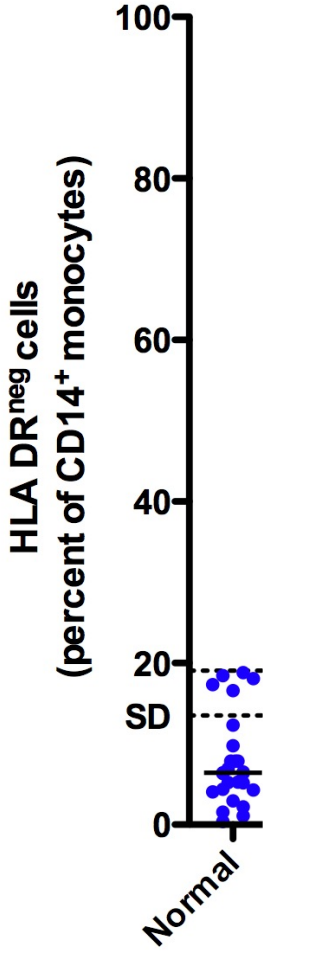
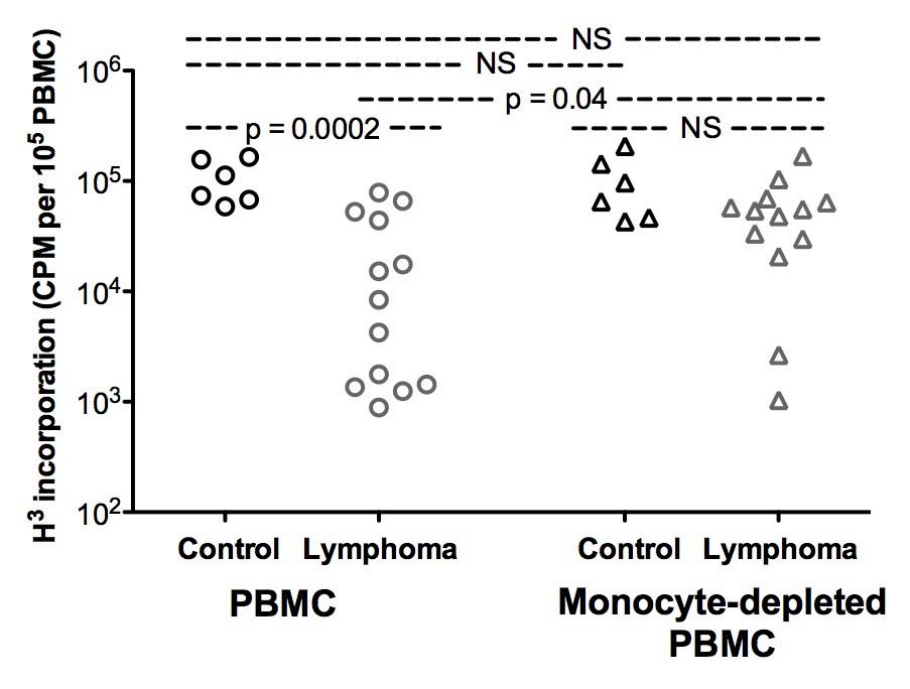


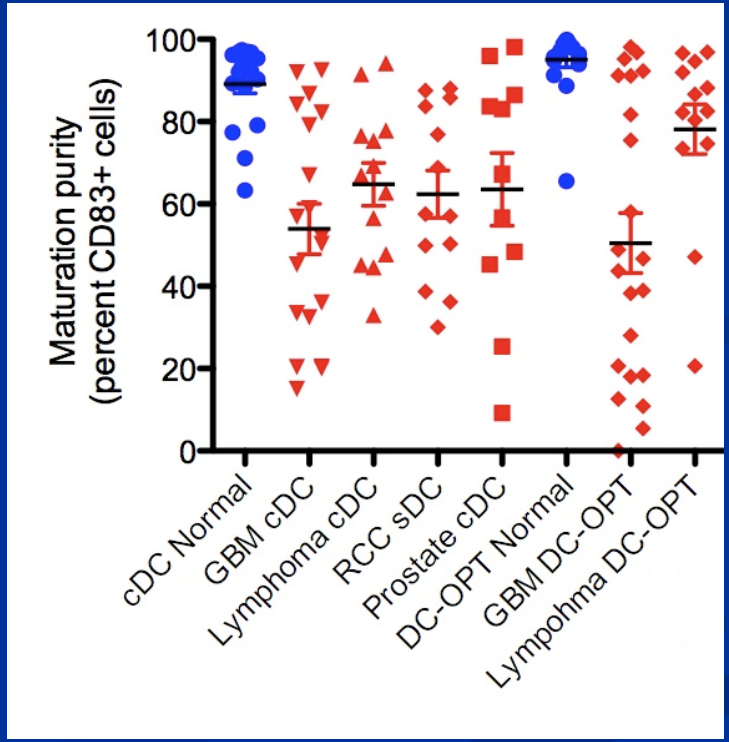
Disease

$CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes mechanisms of immunosuppression

Inhibition of T cell proliferation



Unable to fully differentiate into mature DCs



Gustafson MP, Lin Y, et al, Neuro-Onc. 2010; 12:631-44.
Vuk-pavlovic, et al, Prostate 2010; 70: 443-55.
Lin Y, Gustafson MP, et al, Submitted manuscript.

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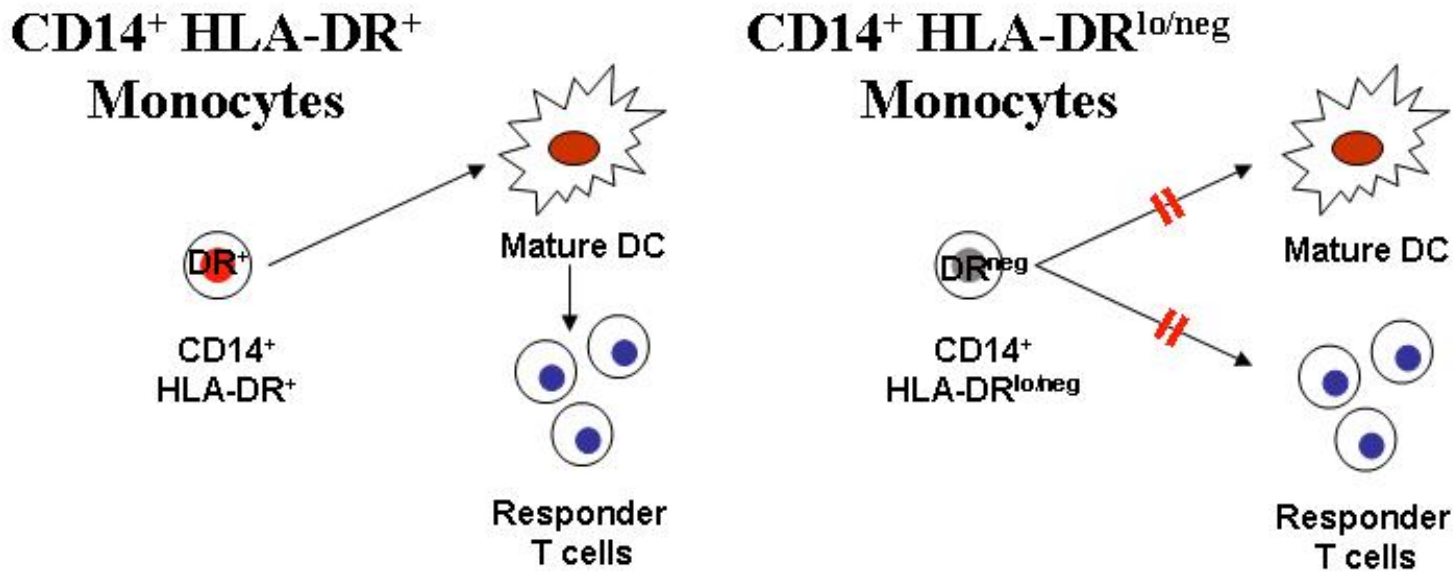

CD14+ HLA-DR+
Monocytes
DR+
CD14+
HLA-DR+
Mature DC
Responder
T cells
CD14+ HLA-DRlo/neg
Monocytes
DRneg
CD14+
HLA-DRlo/neg
Mature DC
Responder
T cells

$CD14^{+}HLA\text{-}DR^{lo/neg}$ in other clinical settings

- Found in melanoma, ovarian cancer, and hepatocellular carcinoma.

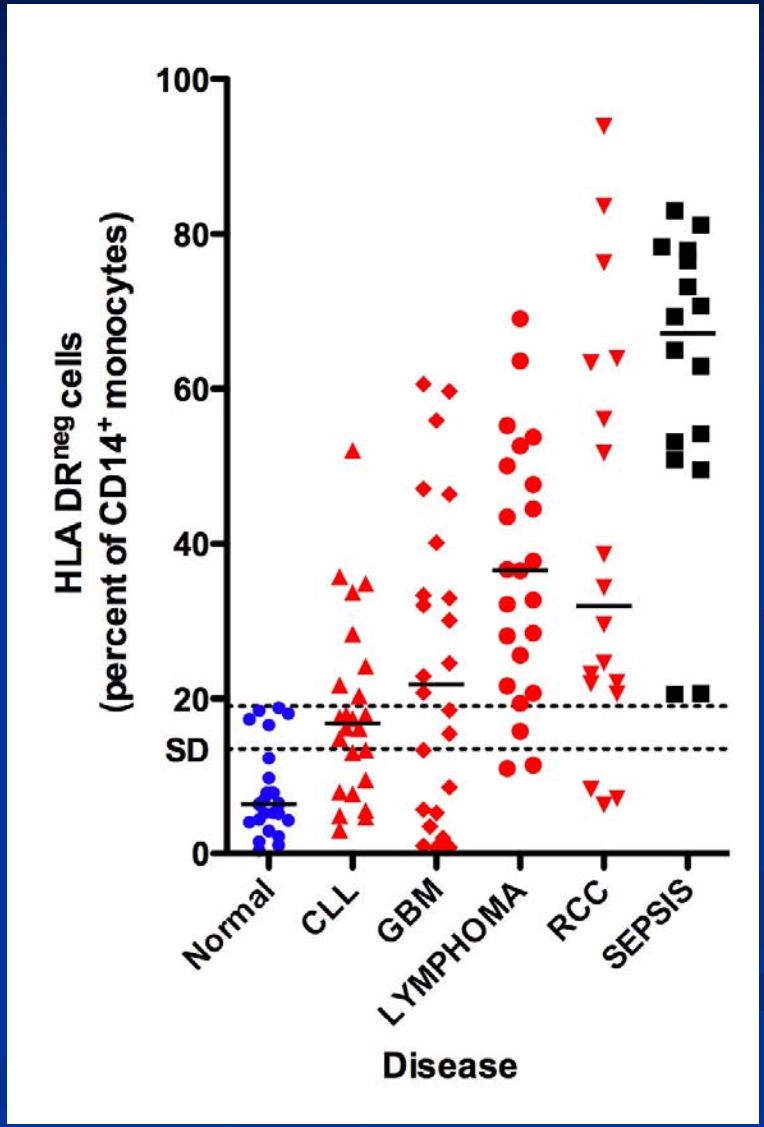
  Valenti R, Cancer Res. 2006;

  Loercher AE, J. Immunol. 1999;

  Hoechst B, Gastroenterology, 2008.

- Associated with sepsis, acute pancreatitis, liver failure, burns, and trauma.
- Correlated with survival in sepsis.

  Cheadle WG, Am. J. Surg. 1993; Wakefield CH, et al, Br. J. Surg. 1993; van den Berk JM, et al, Transplantation 1997; Lekkou A, et al, Clin. Diag. Lab. Immunol. 2004.



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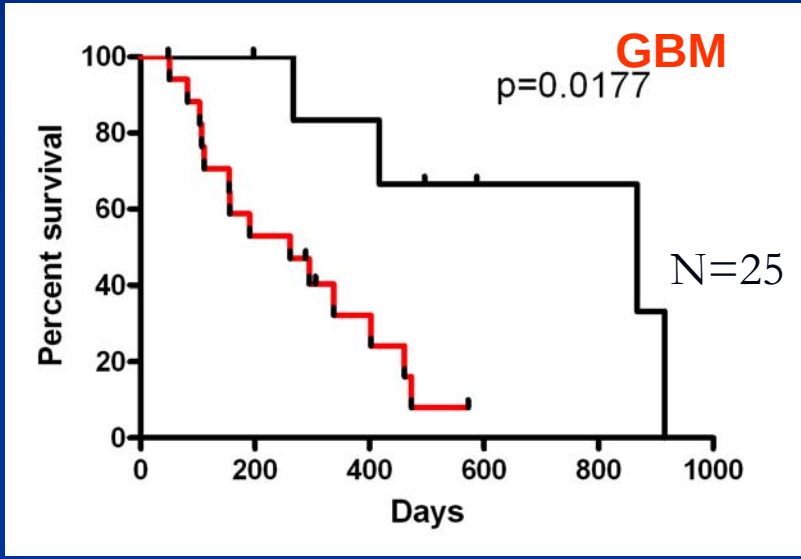
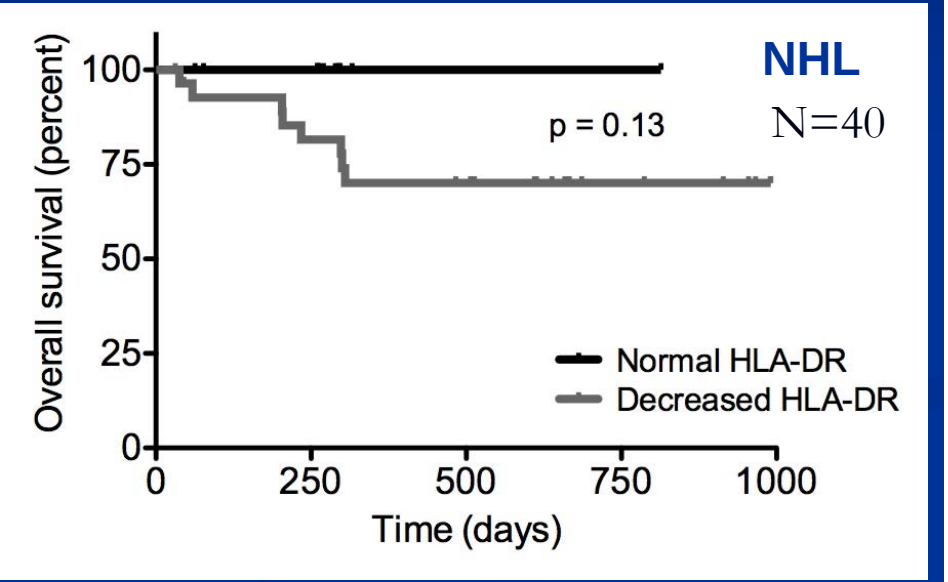
Loss of HLA-DR on CD14+ cells is a prognostic factor in cancer patients





Normal HLA-DR + 1 other phenotype

Abnormal HLA-DR + 1 other phenotype

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Summary

- Immunophenotyping by flow cytometry and multiparameter analysis will continue to be extremely important in characterized baseline immunity in patients.
- Bioinformatics approach is likely to yield new relationships between immune cells.
- $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes are elevated in every cancer type that we've analyzed.
- $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes inhibit T cell proliferation and cannot fully mature into potent DCs.
- The combination of $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes and other phenotypes are prognostic; independent of therapy.

Implications

- The presence of $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes may identify potential responders/non-responders on patients receiving vaccines or other immunotherapeutic approaches.

- Mechanisms of immunosuppression/ immunoparalysis are very similar in cancer patients and in infection/sepsis patients.

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