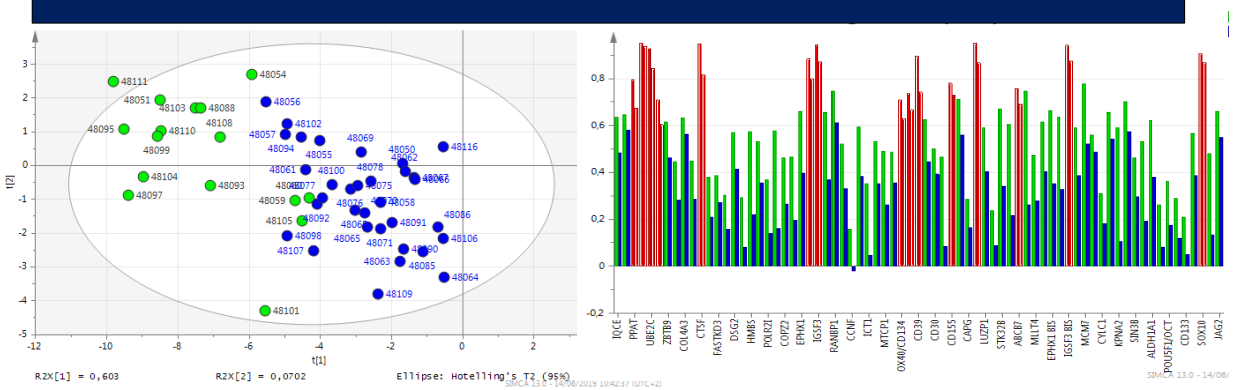
Gene Expression Profiling Test and Immunoscoring for the Molecular Subtyping of High-risk Malignant Melanoma of inflammatory Phenotype

- Metastatic relapse and the likelihood of death among patients with early stage melanoma vary depending on key prognostic variables (Breslow depth, ulceration, mitotic rate, and lymph node involvement), which make up the current AJCC system (1). While patients with resected melanoma are carefully monitored for recurrence by physical examinations, blood tests, and imaging studies (2), we have no means of determining within each staging group who and when they will relapse.
- PBM's scientists reported that endogenous inflammatory factors such as interleukin (IL)-1beta and IL-18 promote experimental melanoma metastasis via vascular endothelial growth factor (VEGF)-induced VLA-4 integrin (3-6) and that IL-18 regulates human melanoma VLA-4 integrin activation through a hierarchized sequence of inflammatory mediators (7). Next they determined signature genes from human melanoma cell lines given soluble VCAM-1 and IL-18, which in turn allowed the identification of primary and metastatic lesions from melanoma patients with inflammation-dependent and –independent phenotypes (8).
- VCAM-1/IL-18-dependent melanoma genes represent a panel of clinically-verified genes of help for sorting melanoma lesions with inflammation-dependent and independent phenotypes. In addition, signature genes in this biomarker panel also defines melanoma with a "liver prometastatic reaction responsive" phenotype. Moreover, distinct melanoma stem cell gene expression patterns were detected for metastatic lesion with and without inflammatory phenotype (recent unpublished data), suggesting that different cancer stem-like cells are operating in these melanoma subtypes. Furthermore, immune checkpoint modulator genes also showed distinct expression patterns in melanoma lesions with inflammation-dependent and independent phenotypes, suggesting their potential interest for better identifying patients responding to immune checkpoint inhibitors (clinical validation now in development). Altogether, VCAM-1/IL-18-dependent melanoma genes and their specific melanoma stem cell and immune checkpoint modulator gene expression patterns are associated with patients with short survival (by multivariate COX regression analysis) compared to patients having a melanoma without this particular phenotype.
- Because inflammation promotes metastatic melanoma progression (9) and upregulates the immunosuppressive microenvironment associated to immune checkpoint pathway activation in effector T cells (10), PBM's scientists have developed novel and sensitive laboratory tests for the detection of primary and metastatic melanoma lesions using inflammation-dependent metastasis and immunosuppressant molecular pathways.

By using **SIMCA (**a well-established advanced multivariate data **analytics** and visualization program), we verified that 37 IL-18/soluble VCAM-1-dependent genes sort patients developing melanoma subtypes with and without inflammation-dependent prometastatic reaction-sensitive phenotypes.



Left Figure: Melanoma with (Green) and without (Blue) inflammatory phenotypes.

Right Figure: In red are genes that contribute most to the segregated distribution of melanomas with and without inflammatory phenotypes.

- Laboratory tests are based on TaqMan Low Density Arrays allowing a relative quantitation of target genes. Next, the expression level and transcriptional association pattern of gene subsets are studied in the array to accurately determining inflammation-dependent and independent melanoma phenotypes with the help of the following gene categories:
  - 1) Genes defining melanoma lesions with and without IL-18/VEGF/VLA-4 phenotype;
  - 2) Genes whose expression changes only occurred during melanoma cell response to proinflammatory mediators; and
  - 3) Cancer stem cell genes and immune checkpoint modulator genes from melanoma lesions with and without IL-18/VEGF/VLA-4 phenotype.

- A verification of transcriptional results can be obtained with the help of the **Inflammation-Dependent Melanoma-immunoscore**™. This is a combined immuno-dermato-pathologic approach to determine clinically-validated biomarkers of inflammation-dependent and independent melanoma cells. The detection kit characterizes the number, density, and distribution of biomarker-positive melanoma cells in the core of the primary and metastatic and in the invasive margin using a combination of immunohistochemistry testing and automated digital pathology. Altogether, the scoring results of the test provide a scientific basis for:
  - **Molecular subtyping of inflammation-dependent and independent melanomas**, their specific prometastatic activation pathway and their associated cancer stem cell and immune checkpoint gene profiles.
  - **Molecular subtyping of melanomas with liver prometastatic reaction responsive phenotype**, whose tumor cells are prone to produce hepatic metastases.
  - **Treatment guidance that may improve patient outcome** based on a better understanding of the inflammation-dependent prometastatic activation pathway and its associated cancer stem cell phenotype in early and advanced stage cutaneous and visceral melanomas. Results may also help to predict response level to immunotherapy in some patients.

References

1. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–206.
2. www.nccn.org
3. Vidal-Vanaclocha F, Amezaga C, Asumendi A, Kaplanski G, Dinarello CA. Interleukin-1 Receptor Blockade Reduces the Number and Size of Murine B16 Melanoma Hepatic Metastases. Cancer Res 1994; 54: 2667-2672.
4. Anasagasti MJ, Olaso E, Calvo F, Martín JJ, Mendoza M, Bidaurrazaga J, and Vidal-Vanaclocha F. Interleukin-1 (IL-1)-Dependent and -Independent Mouse Melanoma Metastases. J Natl Cancer Institute 1997;89:645-651.
5. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martín JJ, Carrascal T, Walsh P, Reznikov LL, Kim S-H, Novick D, Rubinstein M, and Dinarello CA. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Nat Acad Sci USA 2000; 97: 734-39.
6. Carrascal T, L. Mendoza, M. Vacarcel, C. Salado, E. Egilegor, N. Telleria, F. Vidal-Vanaclocha and Charles Dinarello. Interleukin/18 binding protein reduces B16 Melanoma Hepatic Metastasis by neutralizing the adhesiveness and growth factors of sinusoidal endothelial cell. Cancer Res 2003; 63:491-7.
7. Valcárcel M, Carrascal T, Crende O, Vidal-Vanaclocha F. IL-18 Regulates Melanoma VLA-4 Integrin Activation through a Hierarchized Sequence of Inflammatory Factors. J Invest Dermatol 2014;134:470-80.
8. Crende O, Sabatino M, Valcárcel M, Carrascal T, Riestra P, López-Guerrero JA, Nagore E, Mandruzzato S, Wang E, Marincola FM, Vidal-Vanaclocha F. Metastatic lesions with and without interleukin-18-dependent genes in advanced-stage melanoma patients. Am J Pathol 2013;183:69-82.
9. Vidal-Vanaclocha F, Mendoza L, Telleria N, Salado C, Valcarcel M, Gallot N, Carrascal T, Egilegor E, Beaskoetxea J, Dinarello. Clinical and experimental approaches to the pathophysiology of interleukin-18 in cancer progression. Cancer Metastasis Rev 2006; 25:417-34.
10. Landsberg J, Kohlmeyer J, Renn M, Bald T, Rogava M, Cron M, Fatho M, Lennerz V, Wölfel T, Hölzel M, Tüting T. Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. Nature 2012;490(7420):412-6.
11. Crende O, Valcárcel M, Vidal-Vanaclocha F. Cancer stem cell signature genes in inflammation-dependent and independent metastatic lesions from advanced-stage melanoma patients (publication pending).
12. Crende O, Valcárcel M Vidal-Vanaclocha F. Gene Expression Subtyping Defines Malignant Melanoma with Liver Prometastatic Reaction Responsive Phenotype. (publication pending).
13. Crende O, Valcárcel M, Vidal-Vanaclocha F. Primary and Metastatic Melanomas with and without Oxidative Stress-Dependent Signature Genes (publication pending).