Genedata Profiler™

Case Study IMMUNO-ONCOLOGY

Recent advances in cancer immunotherapy such as ipilimumab (Yervoy®, Bristol-Myers Squibb), pembrolizumab (Keytruda®, Merck & Co.) and nivolumab (Opdivo®, Bristol-Myers Squibb) have shown unprecedented success in some advanced cancer patients, validating decades of work in understanding the molecular and cellular mechanism of the antitumor immune response.[1] However, many challenges remain for designing successful and efficient clinical trials for new immuno-oncology therapies, in particular[2]:

- refining endpoints appropriate to the mechanism of action of the particular immunotherapy strategy (for example, irRECIST), by including molecular biomarkers;
- selecting patients eligible for the clinical trial by using prognostic, predictive, and toxicology biomarkers to optimize the clinical benefit of the therapy;
- prioritizing the most rational immunotherapy combinations for clinical development by using biomarkers of primary or secondary resistance.

Consequently, there is an increased need for profiling of patients using a variety of high-throughput technologies from next-generation sequencing (NGS), through proteomics to imaging. The complexity of the technologies and tools for omics analysis presents several challenges for profiling patients in immuno-oncology (I-O) studies. An advanced infrastructure is needed to process, manage and analyze multi-omic data from different technology platforms, from pre-clinical and clinical experiments in conjunction with clinical data, to extract actionable outcomes while ensuring regulatory compliance.[3]

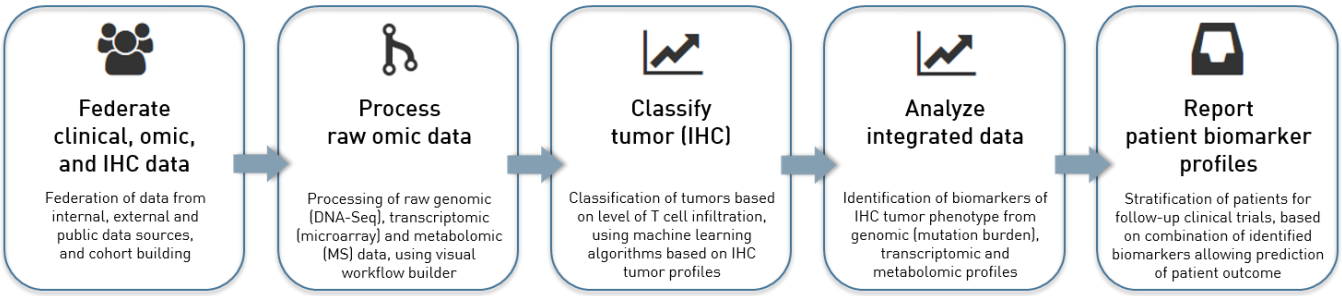

Figure 1: I-O biomarker identification workflow in Genedata Profiler.

Genedata Profiler™ enables translational and clinical researchers to overcome these clinical trial challenges. In a recent I-O clinical trial, scientists using Genedata Profiler followed the process illustrated in Figure. 1 and detailed in this case study to analyze data from 45 patients, and identified predictive biomarkers by integrating genomic, transcriptomic, and metabolomic data, and immunohistochemistry (IHC) tumor profiles. The biomarkers enable further definition of tumor subtypes and thus identification and selection of patient profiles that will benefit from the I-O therapy, consequently improving the outcome of the treatment.

Genedata solutions in silico

Integration of clinical and omic data

The sample and data management system in Genedata Profiler establishes a working space for a research project (study) which contains all the raw and processed data, data processing pipelines, and statistical analysis, and captures scientific insights and clinical implications. The study structure of Genedata Profiler facilitates collaboration among scientists and allows integration of external and internal data, and data from public sources.

DNA-Seq data for this analysis was generated externally by a CRO and provided as FASTQ files, which were linked from an FTP server directly into the study in Genedata Profiler, without the need to download the data locally. The clinical annotations of the patients were uploaded into Genedata Profiler, and metabolomics (LC-MS), IHC and transcriptomics (microarray) datasets of corresponding patients were linked into the repository.

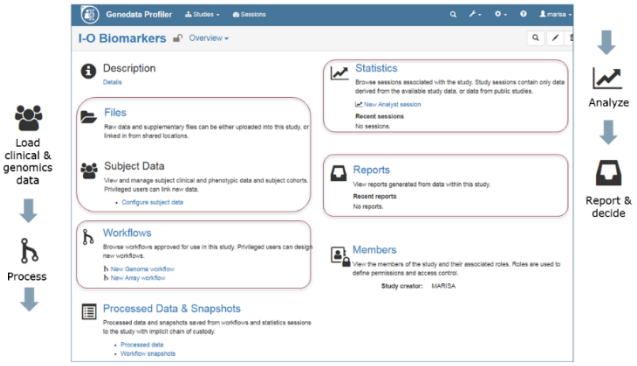

Figure 2: Study-centric data and project management system of Genedata Profiler. Chain of custody is maintained in Genedata Profiler to ensure that scientific insights can be validated by inspecting data at all levels of processing, at any time.

Raw omic data processing

Genedata Profiler enables users to visually develop automated data processing workflows for multiple data types. Comprehensive data processing workflow templates for all major NGS, mass spectrometry (MS) and microarray applications are provided with Genedata Profiler, following state-of-the-art data analysis practices. Genedata Profiler maintains full chain of custody, ensures consistent data processing, allows visual validation of the findings in the Genome Browser (NGS) and in the Ion Map (MS), and minimizes the cost of data handovers.

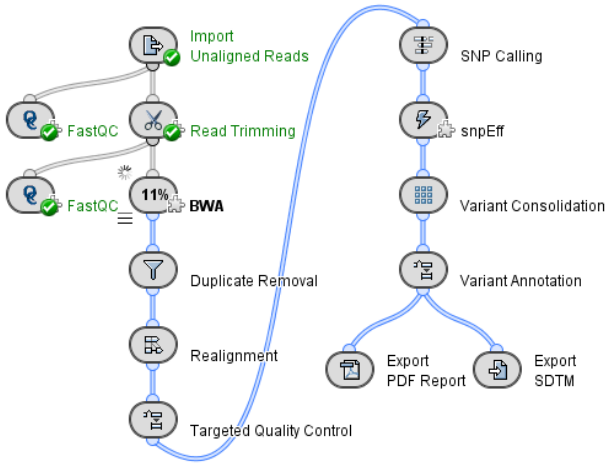

Figure 3: DNA-Seq processing workflow. Green check marks indicate completed processing steps. The BWA processing step is currently running and is 11% complete.

Using workflow templates, pipelines to process the 45 biopsy profiles at the genomic (DNA-Seq), transcriptomic (microarray) and metabolomic (MS) levels were set up. The established pipelines were launched and the data was processed, quality-controlled and analyzed.

Integrative data analysis to classify tumors and identify biomarkers

A comprehensive list of normalizations and transformations is available in Genedata Profiler. Out of the box, the software integrates data from all sources and provides data analytics tools including machine learning algorithms.

Tumor classification based on immunological response

Based on T cell infiltration, tumors can be classified into immunologically ignorant (cold), immunologically responsive (hot) and those with intermediate phenotype (warm). Immunologically responsive tumors are more likely to respond to I-O therapies, including checkpoint blockade antibody therapies. Current classification strategies are tumor type-specific, subjective and not standardized.[4–7]

Subjective assignment of 45 tumors into the three categories based on IHC profiling of five cellular markers (CD3, CD8, CD68, FOXP3, PD-1) was refined in this study by unbiased analysis of IHC profiles using machine learning algorithms (decision tree, support vector machine and k-nearest neighbors).

Two markers, CD3 and CD8, were identified as sufficient to classify more than 86% of samples according to the tumor infiltration level.

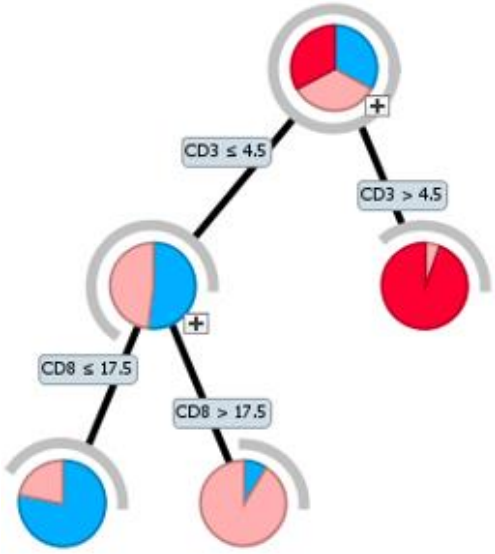

Figure 4: Decision tree classification of tumors based on their IHC profile. Red: hot tumor, pink: warm tumor, blue: cold tumor.

Kaplan-Meier survival analysis of patients with tumors of different T cell infiltration levels

It has been shown that the level of T cell infiltration of tumors is indicative of patient outcome for I-O treatments in some tumor types.[8,9] The patients were stratified according to their IHC profiles and a Kaplan-Meier survival analysis was performed. The analysis indicated that this stratification could improve prediction of individual patient outcome.

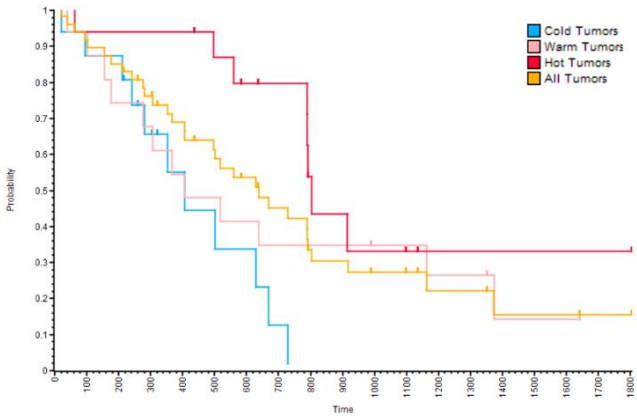

Figure 5: Kaplan-Meier survival analysis shows predicted outcome for the entire patient cohort (in orange). Significantly improved predictions for individual patient strata based on tumor IHC profiling are shown in red, pink and blue.

Identification of tumor gene expression and metabolite signatures to complement mutation burden for patient stratification

Following patient stratification by IHC tumor classification, analysis of genomics, transcriptomics and metabolomics was used to profile tumors to identify biomarkers corresponding to T cell infiltration. Scaling and z-transformation of the transcriptomics and metabolomics datasets was performed prior to their integrative analysis. The genomic data was used to calculate tumor mutation burden. The analysis confirmed previous findings that hot tumors have the highest mutation burden.[10,11]

An unbiased classification approach using tools from the extensive data analytics toolbox in Genedata Profiler (support vector machine using Fisher LDA and k-nearest neighbors as ranking methods) identified six biomarkers (three genes and three metabolites) for the level of T cell tumor infiltration.

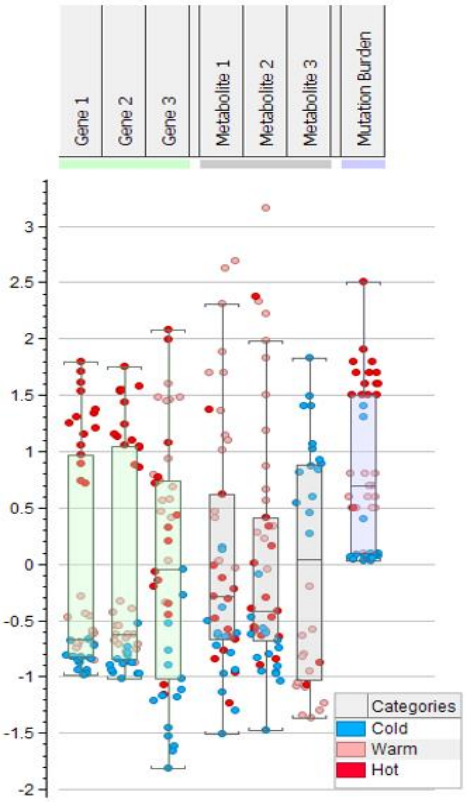

Figure 6: Column plot illustrating identified biomarkers. The levels of gene expression and recorded metabolites are colored according to T cell infiltration level.

Summary

Machine learning algorithms in Genedata Profiler were used to produce unbiased classification and stratification of tumors, based on T cell infiltration levels. Genomics, transcriptomics, and metabolomics were integrated and analyzed to identify biomarkers for I-O tumor category. This set of biomarkers enables identification of a patient profile that predicts benefit from the studied I-O therapy, improving the outcome of the treatment for the targeted patient subpopulation. Follow-up studies are needed to validate the insights and to identify potential clinical and commercial applications of the identified transcriptomic and metabolomic markers to advance targeted therapies for cancer.

The complexity of tumor-immune interactions, acquired resistance due to gene mutation or rearrangement, and the vast number of potential combination therapies, underscore the need for the implementation of translational immunology strategies that will become increasingly vital to refine research strategies and accelerate the development and approval of potential new I-O therapies.

Genedata Profiler can be licensed for use in-house or deployed via a consulting project through Genedata Data Science Services to generate valuable scientific and business insights.

References

1. Yuan J, Hegde PS, Clynes R, et al. Novel technologies and emerging biomarkers for personalized cancer immunotherapy. *J Immunother Cancer.* 2016 Jan 19;4:3. doi: 10.1186/s40425-016-0107-3.
2. Emens LA, Butterfield LH, Hodi FS Jr, Marincola FM, Kaufman HL. Cancer immunotherapy trials: leading a paradigm shift in drug development. *J Immunother Cancer.* 2016 Jul 19;4:42. doi: 10.1186/s40425-016-0146-9.
3. Siu LL, Conley BA, Boerner S, LoRusso PM. Next-generation sequencing to guide clinical trials. *Clin Cancer Res.* 2015 Oct 15;21(20):4536–44. doi: 10.1158/1078-0432.CCR-14-3215.
4. Galon J, Mlecnik B, Bindea G, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol.* 2014 Jan;232(2):199–209. doi: 10.1002/path.4287.
5. Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying cancers based on T cell infiltration and PD-L1. *Cancer Res.* 2015 Jun 1;75(11):2139–45. doi: 10.1158/0008-5472.CAN-15-0255.
6. Sznurkowski JJ, Zawrocki A, Emerich J, Biernat W. Prognostic significance of CD4+ and CD8+ T cell infiltration within cancer cell nests in vulvar squamous cell carcinoma. *Int J Gynecol Cancer.* 2011 May;21(4):717–21. doi: 10.1097/IGC.0b013e3182131f36.
7. Pagès F, Galon J, Dieu-Nosjean MC, Tartour E, Sautès-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene.* 2010 Feb 25;29(8):1093–102. doi: 10.1038/onc.2009.416. Epub 2009 Nov 30.
8. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer.* 2012 Mar 15;12(4):298–306. doi: 10.1038/nrc3245.
9. Erdag G, Schaefer JT, Smolkin ME, et al. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res.* 2012 Mar 1;72(5):1070–80. doi: 10.1158/0008-5472.CAN-11-3218. Epub 2012 Jan 19.
10. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med.* 2017 Apr 19;9(1):34. doi: 10.1186/s13073-017-0424-2.
11. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015 Apr 3;348(6230):124–8. doi: 10.1126/science.aaa1348. Epub 2015 Mar 12.