New biological markers in MM

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A TARGETED SEQUENCING APPROACH IN MULTIPLE MYELOMA REVEALS A COMPLEX LANDSCAPE OF GENOMIC LESIONS THAT HAS IMPLICATIONS FOR PROGNOSIS

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Background: Next-generation sequencing (NGS) studies have shown that multiple myeloma is a heterogeneous disease with a complex subclonal architecture and few recurrently mutated genes. The analysis of smaller regions of interest in the genome ("targeted studies") allows interrogation of recurrent genomic events with reduces complexity of downstream analysis at a lower price.
Aims: Here, we performed the largest targeted study to date in multiple myeloma to analyze gene mutations, deletions and amplifications, chromosomal copy number changes and immunoglobulin heavy chain locus (IGH) translocations and correlate results with biological and clinical features.
Methods: We used Agilent SureSelect cRNA pull down baits to target: 246 genes implicated in myeloma or cancer in general in a mixed gene discovery/confirmation effort; 2538 single nucleotide polymorphisms to detect amplifications and deletions at the single-gene and chromosome level; the IGH locus to detect translocations. We sequenced unmatched DNA from CD138-purified plasma cells from 418 patients with multiple myeloma at diagnosis, with a median follow-up of 5.3 years. We sequenced at an average depth of 337x using Hiseq2000 machines (Illumina Inc.). We applied algorithms developed in-house to call genomic events, filtering out potential artifacts and germline variants. We then ranker each event on its likelihood of being "oncogenic" based on clustering, recurrence and cross-reference with the COSMIC database.
Results: We identified 2270 gene mutations in 412/418 patients, and of those 688 were oncogenic. 342 patients harbored at least one oncogenic mutation. 215/246 genes showed at lease one likely somatic mutation, but only 106 showed at least one oncogenic mutation. 63% of oncogenic mutations were accounted for by the top 9 driver genes previously identified (KRAS, NRAS, TP53, FAM46C, BRAF, DIS3, TRAF3, SP140, IRF4), implying our gene discovery effort did not identify novel mutated genes. We included deletion of tumor suppressors, amplification of oncogenes, chromosomal copy number changes and IGH translocations for a total of 76 variables, so that 413/418 patients showed at least one informative driver genomic event, (median 4/patient). We investigated pairwise associations between events and found significant correlations, such as TP53 mutations and del(17p), CYLD mutations and del(16), FAM46C mutations and del(1p), SF3B1 mutations and t(11;14). Hotspots mutations of IRF4 lysine p.123 showed an inverse correlation with a hyperdiploid karyotype and del(16) as opposed to other missense mutations scattered along the gene, which has pathogenic implications. Survival was negatively affected by the cumulative burden of lesions in an almost linear fashion, with median survival of 10.97 and 4.07 years in patients with <=2 or >=7 lesions respectively, and this was independent of the nature of the genomic events. Given the heterogeneity and complex interplay of the variables we fitted a cox-proportional hazard model to predict survival. We found that mutations in TP53, amplifications of MYC, deletions of CYLD, amp(1q), del12p13.31 and del17p13 where the only significant events, all promoting shorter survival. In particular, TP53 mutations and deletions, often co-occurring, had an additive effect so that carriers of both showed a dismal survival of 17 months (Figure 1).

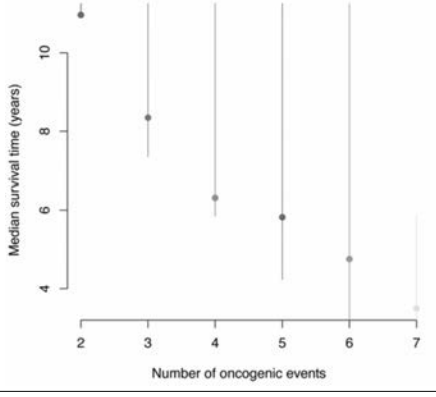

Figure 1.

Summary/Conclusions: Due to the complex genomic landscape in MM, a discovery effort still requires large studies to derive significant associations. We conclude that a targeted sequencing approach may provide prognostic models and give insights into myeloma biology.

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A NEW MULTIPLE MYELOMA CLASSIFICATION SYSTEM THAT CORRELATES TO DISEASE STAGE AND PROGNOSIS - INDICATION OF REVERSIBLE PHENOTYPIC PLASTICITY AS A HALLMARK

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Background: Today's diagnostic tests for multiple myeloma (MM) reflect the criteria of the updated WHO classification based on biomarkers and clinicopathologic heterogeneity.
Aims: To that end, we propose a new biological subtyping of myeloma plasma cells (mPC) by B-cell subset associated gene signatures (BAGS), from the normal B-cell hierarchy in the bone marrow (BM). Here we document the prognostic and biological value of subtyping, as shown for DLBCL (JCO 2015 Apr 20; 33:1379).
Methods: We combined FACS and GEP to generate BAGS classifiers for the normal BM subsets: PreB-I, PreB-II, immature (Im), naive (N), memory (M) and PC. Construction was based on median-centred probe sets from the BM data using regularized multinomial regression with six discrete outcomes representing BAGS, by a total of 55 genes varying from 15-24 per subtype. Each patient underwent BAGS assignment according to the highest predicted probability score above 0.45 or was otherwise unclassified. The impact of BAGS was analyzed using six clinical cohorts, gathered across geographical regions, time eras, and sampling methods. The analysis estimated subtype frequencies and included a prognostic meta-analysis of 926 patients treated with high dose melphalan as first line therapy in 3 prospective trials: UAMS, HOVON65/GMMG-HD4, MRC Myeloma IX data with the Affymetrix U133 plus 2.0 microarray data available from myeloma PC samples. To compensate for cohort-wise technical batch effects, each cohort was median centred and adjusted probe set-wise to have same variance as the BM data.
Results: *Validation of the normal B-cell subset phenotypes.* Normalized histograms of the fluorescence intensities (FI) of CD markers based on merged multiparametric flow cytometry reanalysis of pure sorted populations resulting from seven independent sorting procedures documented high purity. Principal component analysis (PCA) of the FI for each sorted cell in all samples documented specificity. Surface markers, transcription factors, and B-cell differentiation–specific genes were identified through a literature review, and their expression across subsets was evaluated. The most varying probe sets were included in an unsupervised hierarchical clustering analysis, supporting the biological differences. *Validation of MM patients subtyping by prognosis.* The resultant tumor assignments exhibited very similar BAGS subtype frequencies, across 1302 individual MM cases from 4 different cohorts. The 5 BAGS subtypes of 926 MM cases were significantly associated with overall ($P=5.2x10^{-8}$) and progression free ($P=1.5x10^{-6}$) survival in a meta-analysis of patients in the 3 clinical trials. The major impact was observed within the PreB-II and M subtypes conferred with significant increased ISS stage III and inferior prognosis compared to the Im, N and PC subtypes. Cox proportional hazard meta-analysis showed that the five BAGS subtypes added significant and independent prognostic information to the TC classification system and plasma Beta-2 microglobulin level. In parallel we found significant correlation between the PreBII subtypes and the proliferation index, risk profiling ($P<0.0001$) and Beta-2 microglobulin ($P<0.001$).
Summary/Conclusions: We have documented patient specific mPC differences with prognostic impact in support of reversible phenotypic plasticity in MM. This observation provides a new model for generating insight into the stages of clonal plasticity associated with oncogenesis and dedifferentiation.