E1199

A MULTICENTER, OPEN-LABEL, PHASE I CLINICAL STUDY: SAFETY, EFFICACY, AND PHARMACOKINETICS OF ORAL RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES

K. Ishizawa[12,*], K. Usuki[3], K. Ando[4], Y. Ueda[5], T. Kiguchi[6], N. Uike[78], Y. Onishi[2], H. Iida[9]
[1]Hematology and Cell Therapy, Yamagata University Faculty of Medicine, Yamagata, [2]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai, [3]Department of Hematology, NTT Medical Center Tokyo, Tokyo, [4]Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, [5]Department of Hematology/Oncology, Kurashiki Central Hospital, Kurashiki, [6]Department of Hematology, Chugoku Central Hospital, Fukuyama, [7]Department of Palliative Care Medicine, Saga Medical Center Koseikan, Saga, [8]Department of Hematology, National Hospital Organization Kyushu Cancer Center, Fukuoka, [9]Department of Hematology, National Hospital Organization Nagoya Medical Center, Nagoya, Japan

Background: Rigosertib, a novel phosphoinositide 3 kinase pathway inhibitor, induces G2/M arrest leading to the apoptosis of cancer cells and myeloblasts and is safe for and well tolerated by pts with low, intermediate-1, intermediate-2, or high-risk myelodysplastic syndromes (MDS).
Aims: The aims of the study were to assess the safety, efficacy, and pharmacokinetics of oral rigosertib and to determine the recommended dose (RD) for a Phase II clinical study in Japanese pts with recurrent/relapsed or refractory MDS.
Methods: We conducted a multicenter, open-label, Phase I clinical study of oral rigosertib. The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: 20 or older; ECOG PS of 0 to 2; and no major organ dysfunctions. Rigosertib (280 and 560mg BID) was administered orally in one 21-day cycle (up to cycle 6) that consisted of the 14-day, twice-daily, oral administration term, followed by 7-day monitoring. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results, 2) efficacy as assessed with the International Working Group 2006 criteria, and 3) pharmacokinetics.
Results: Between March 2013 and November 2014, 6 male and 3 female pts (median age: 70; range 52-80) were enrolled. ECOG PS was 0 in 7 pts and was 1 in 2 pts, and 3 and 6 pts were eventually assigned to the 280 and 560mg BID arms, respectively. According to the FAB classification, 4, 2, 2, and 1 pts were categorized to RAEB, RARS, RA, and RAEB-t, respectively. The prognostic factor according to IPSS was Int-1 risk in 4 pts (1 and 3 pts in the 280 and 560mg BID arms, respectively) and was Int-2 in 5 pts (2 and 3 pts in the 280 and 560mg BID arms, respectively). DLT occurred in 1 pt in the 280mg BID arm and in 2 pts in the 560mg BID arm: the former consisted of type 2 diabetes and grade 4 delirium, and the latter grade 5 urinary tract infection and grade 3 prolonged QT interval. Therefore, the RD for a Phase II clinical study in Japanese pts was determined to be 560mg BID. On day 11 of treatment, 1 pt in the 560mg BID arm died of grade 5 urinary infection whose relationship with the investigational drug was rated to “Definite”. The presumed cause of death for this patient was septic shock caused by urinary tract infection. The mean counts of leukocytes, neutrophils, lymphocytes, and reticulocytes in the 280mg BID arm did not decrease along with increases in the number of cycles delivered but decreased slightly in the 560mg BID arm. Any changes of note were not found in other hematological items. One case of grade 3 neutropenia developed in the 280mg BID arm, and 1 case each of grade 3 laboratory abnormalities—increased alanine aminotransferase, increased aspartate aminotransferase, prolonged QT interval, neutropenia, and decreased hemoglobin—occurred in the 560 BID arm. The hematological remission rate was 11.1% (1 marrow CR; 1/9 pts), and the hematological improvement rate was 11.1% (1 HI-P; 1/9 pts). Among the PK parameters, inter-individual variability was observed in the C_{max} and AUC. However, changes suggesting the accumulation of rigosertib during repeated oral administration (e.g., consistent increases in the C_{max} and AUC) were not found.
Summary/Conclusions: The present chemotherapy regimen of oral rigosertib was well tolerated. Our study indicates that the RD for a Phase II clinical study is 560mg BID in Japanese patients with recurrent/relapsed or refractory MDS.

Myeloma and other monoclonal gammopathies - Biology

E1200

NON-OVERLAPPING PROMOTER AND SUPERENHANCER DRIVEN PROCESSES SUPPORT MYELOMA CELL GROWTH AND SURVIVAL VIA DISTINCT REGULATORY AXES

M. Fulciniti[1,*], C. Lin[2], M. Samur[1], H. Avet-Loiseau[3], K. Anderson[1], J. Bradner[4], N. Munshi[1]
[1]Dana Farber Cancer Institute, Boston, [2]Baylor college of medicine, Houston, United States, [3]Cancer research center, Toulouse, France, [4]Novartis, Boston, United States

Background: We have previously reported that E2F1 and its heterodimerization partner DP1 promote MM tumor proliferation both *in vitro* and in vivo; and observed an inverse correlation between their expression and patient survival suggesting a role in MM pathogenesis. Moreover, E2F functional impairment by a dimerization inhibiting stapled peptide significantly affects myeloma tumor cell growth while sparing effect on normal components of bone marrow as well as normal plasma cells, suggesting an E2F dependency in MM cells.
Aims: In this study, our aim was to defined the regolatory landscape of E2F in MM to better understand how E2F1 and DP1 drive myeloma cell proliferation; and to define the relationship between promoter proximal transcription factor-associated gene expression and super-enhancer-driven transcriptional programs.
Methods: We integrated genetic perturbation with functional omics to define E2F role in MM. Global occupancy of E2F1 and DP1 in MM was evaluated by ChIP-seq analysis. E2F1 and DP1 genomic localizations were then integrated to MM reference epigenome. Enhancers and super-enhancers were mapped using ROSE2 (github.com/bradnerlab/pipeline). Read densities were calculated using bamliquidator (github.com/BradnerLab/pipeline/wiki/bamliquidator).
Results: Integration of E2F1 and DP1 genomic localization to MM reference epigenome revealed specific co-occupancy of the factors at promoters of active genes marked by H3K4me3, with a strong positive correlation between E2F and RNA Polymerase II (RNA Pol II) binding at transcription start sites. In contrast, active enhancers, as defined by promoter distal Mediator (MED1) peaks and marked by H3K27ac and BRD4, showed virtually no E2F binding. Prompt by these observations, we explored the transcriptional and functional interrelationship between E2F and BETs to identify their individual contribution to eventual functional effect in MM. Unbiased hierarchical clustering revealed distinct regulatory axes for E2F and BETs, with E2F predominantly localized to active gene promoters of growth/proliferation genes and BETs disproportionately at enhancer- regulated tissue specific genes confirming that these factors establish distinct target gene programs. At the extremes, we found less than 10% of genes were among the top 500 in BRD4 enhancer signal (*i.e.* SE-regulated) and top 500 E2F promoter signal. We hypothesized that the presence of BETs and E2F in distinct regulatory axes divides active genes in MM into those that can be selectively influenced by BET inhibition or E2F perturbation, but not both. In line with this we have observed that dual E2F and BET inhibition is synergistic for MM cell growth, both *in vitro* and in vivo.
Summary/Conclusions: In conclusions, our results highlight the existence of non-overlapping promoter and super-enhancer-associated dependencies in multiple myeloma, suggesting a sequestered molecular control that may be perturbed in cancer with potential for development of a promising therapeutic strategy.

E1201

ANALYSIS OF THE GENOMIC LANDSCAPE OF MULTIPLE MYELOMA HIGHLIGHTS NOVEL CANDIDATE PROGNOSTIC MARKERS AND DISEASE SUBGROUPS

N. Bolli[1,*], G. Biancon[2], M. Moarii[3], S. Gimondi[2], Y. Li[4], C. De Philippis[2], F. Maura[2], V. Sathiaseelan[4], T. Yu-Tzu[5], L. Mudie[4], S. O'Meara[4], K. Raine[4], J.W. Teague[4], A.P. Butler[4], C. Carniti[6], M. Gerstung[7], T. Bagratuni[8], E. Kastritis[8], M.A. Dimopoulos[8], P. Corradini[2], K.C. Anderson[5], P. Moreau[9], S. Minvielle[10], P.J. Campbell[4], E. Papaemmanuil[3], H. Avet-Loiseau[11], N.C. Munshi[5]
[1]Department of Oncology and Onco-Hematology, [2]University of Milan, Milan, Italy, [3]Memorial Sloan Kettering Cancer Center, New York, United States, [4]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [5]Dana-Farber Cancer Institute, Boston, United States, [6]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, [7]European bioinformatics institute, Cambridge, United Kingdom, [8]National and Kapodistrian University of Athens, Athens, Greece, [9]Centre Hospitalier Universitaire de Nantes, [10]Center for Cancer Research Nantes-Angers, Nantes, [11]Centre de Recherche en Cancerologie de Toulouse, Toulouse, France

Background: In multiple myeloma (MM), next generation sequencing (NGS) has expanded our knowledge of genomic lesions, and highlighted a dynamic and heterogeneous composition. Despite a growing number of cases sequenced, the full potential of NGS studies has not been exploited so far.

Aims: We used a custom target pulldown (TPD) approach on a large cohort of MM samples at diagnosis, with homogeneous treatment and long follow-up, to further our understanding of the landscape of driver lesions in MM and how this can be used to improve prognostication and disease classification.
Methods: We used a custom-designed SureSelect pulldown strategy (Agilent Biotechnologies) to target 246 genes implicated in MM or cancer in general; 2538 single nucleotide polymorphisms; the immunoglobulin heavy chain (IGH) locus. We sequenced unmatched DNA from CD138-purified plasma cells from 418 patients with a median follow-up of 5.4 years using Illumina Hiseq2000 machines. We applied algorithms developed in-house to detect driver genomic events, filtering out potential artifacts and germline variants. We then ranked each mutation on its likelihood of being oncogenic.
Results: We identified 197 driver events including gene mutations, aneuploidies and IGH translocations (IGH-Tx), median of 6 per patient. Gene mutations where found in >99% of patients. At least one oncogenic mutation of a known driver gene previously identified (*KRAS, NRAS, TP53, FAM46C, BRAF, DIS3, TRAF3, SP140, IRF4*) was found in 64%, with a long tail of infrequently mutated genes with uncertain significance. Karyotypic class was assigned in 80% of patients, with 9% of hyperdiploid cases also showing an IGH-Tx (mostly t(4;14)). IGH-Tx and aneuploidies dominated the MM genomic landscape, KRAS and NRAS being the only point mutations present in the 15 most frequent driver events. Multivariate analysis by sparse Cox regression highlighted only four driver events with significant prognostic impact for both progression-free (PFS) and overall survival (OS): t(4;14) (HR 1.88, CI 1.25-1.84), amp(1q) (HR2.63, CI 1.92-3.59), del(17p) (HR2.55, CI 1.66-3.92), and rare mutations of *ATP13A4* (HR 0.08, CI 0.01-0.65, mutated in 1.4% of patients). We found a significantly worse prognosis for increasing numbers of driver lesions in each patient (median OS 8.2 *vs* 3.5 years for <5 and>8 driver events, respectively). This was only partially explained by instances of additive effect or interactions between variables, which were very informative but not frequent. To better investigate these findings in the context of the genomic landscape of each case, we applied Bayesian clustering algorithms. The large number of driver events screened led to the identification of three groups: in the largest one, some hyperdiploid and IGH-Tx cases clustered together, suggesting that secondary mutations and CNAs required for tumor progression are often shared between these two subgroups. We then identified two clusters both characterized by significantly lower number of mutations, but with opposing features. One was enriched for IGH-Tx, had the highest number of CNAs overall, showed higher prevalence of amp(1q), del(13), del(17p), *TP53* mutations, and had a shorter median OS of 5.3 years. The other was mostly composed of hyperdiploid cases and showed fewest CNAs and mutations, with a good prognosis (median OS not reached).

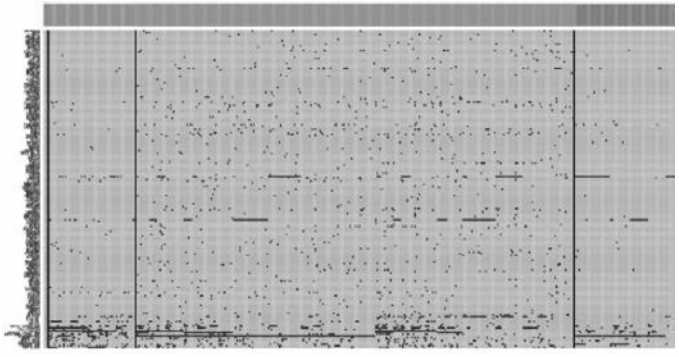

Figure 1.

Summary/Conclusions: We report on the first attempt towards the use of extended tumor genotype for a genomic classification of MM using innovative clustering algorithms. Despite the heterogeneity of the disease, we could identify disease subgroups with a distinct spectrum and number of driver events carrying different prognosis, supporting the introduction of genomics in the clinical approach to MM

E1202

A NOVEL METHOD FOR GENOME-WIDE COPY NUMBER ASSESSMENT FROM TARGETED SEQUENCING DATA AND CLINICAL APPLICATION IN PATIENTS WITH MULTIPLE MYELOMA

G. Ryland[1,*], J. Markham[2], K. Jones[1], E. Aydogan[1], T. Papenfuss[2], M. Chin[3], J. Sive[4], K. Yong[3], M. Prince[5], D. Westerman[1], M. Dickinson[5], P. Blombery[1]
[1]Pathology, [2]Research, Peter MacCallum Cancer Centre, Melbourne, Australia, [3]UCL Cancer Institute, [4]Department of Haemato-Oncology, St Bartholomew's Hospital, London, United Kingdom, [5]Haematology, Peter MacCallum Cancer Centre, Melbourne, Australia

Background: Assessment of gene mutations by next generation sequencing is now standard in patients with haematological malignancy. However, larger chromosomal aberrations (e.g. exon, gene and chromosome level gains and losses) also serve as critical prognostic indicators that guide therapeutic decision making. These larger genetic lesions are typically detected using a separate methodology such as conventional cytogenetics/FISH.
Aims: We aimed to develop and clinically validate a novel method for assessing genome-wide copy number changes using an existing hybridisation-based targeted sequencing panel in order to provide further critical prognostic information in addition to variant-level data without the need for a separate assay.
Methods: A custom Agilent SureSelect capture panel targeting 313 genes of relevance in myeloid and lymphoid malignancies was sequenced on an Illumina NextSeq (paired end 75bp reads) to a mean depth of 700x. An in-house bioinformatics pipeline was created to analyse probe counts from on-target and off-target reads, which also corrects for biases introduced during DNA enrichment and sequencing by normalisation to a pooled reference comprising 10 normal controls. Three metrics for copy number calling were tested including a per-mutation-based statistic from circular binary segmentation, weighted mean and variance for the bins in each segmented region, and an MLPA-like test using read count ratios compared to controls. An interactive web-based graphical user interface was developed to visualise both large-scale and exon level amplification and deletions.
Results: We validated the approach on 45 samples from patients with multiple myeloma (predominantly advanced disease) with known copy number status as determined by conventional cytogenetics, FISH and MLPA. Our novel method detected numerous copy number changes that were outside the targeted region (through genome-wide mapping and analysis of off-target reads) such as del(1p) in 12 patients, gain(1q) in 15 patients and MYC amplification in 5 patients. Moreover our method was able to interrogate and resolve the complexity of changes on del(1p) including isolated deletions of FAM46C, CDKN2C and FAF1. Of 25 patients with a TP53 mutation, 20 had concomitant del(17p) detected by our assay, while 1 case had a del(17p) without mutation; both monoallelic and biallelic TP53 aberration was associated with poor survival. Other findings in this cohort include frequent DIS3 mutations in patients with monosomy 13 and novel oncogenic copy number changes such as the high level amplification of KRAS in 1 case.
Summary/Conclusions: We have developed and demonstrated utility of a reliable workflow for genome-wide copy number assessment that can be implemented using existing targeted short read sequencing data, greatly extending the utility of this technology beyond the identification of mutations in patients with haematological malignancy. In the context of myeloma this can be used to report clinically relevant changes including deletions of 1p and 17p, and gains of 1q and 8q, as well as novel numerical chromosome aberrations.

E1203

THE MULTIPLE MYELOMA GENOME PROJECT: DEVELOPMENT OF A MOLECULAR SEGMENTATION STRATEGY FOR RISK STRATIFICATION OF MULTIPLE MYELOMA

K. Mavrommatis[1], B. Walker[2], M. Samur[3], F. Towfic[4], C. Ashby[2], C. Wardell[2], M. Bauer[2], M. Ortiz[5], E. Flynt[4], Z. Yu[4], M. Amatangelo[4], M. Trotter[5], H. Avet-Loiseau[6], G. Jackson[7], K. Anderson[3], A. Thakurta[4], N. Munshi[3], G. Morgan[2,*]
[1]Celgene Corporation, San Francisco, [2]Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, [3]Dana Farber Cancer Institute, Harvard Medical School, Boston, [4]Celgene Corporation, Summit, United States, [5]Celgene Corporation, Seville, Spain, [6]Centre de Recherche en Cancérologie de Toulouse Institut National de la Santé et de la Recherche Médicale, Toulousse, France, [7]Newcastle University, Newcastle, United Kingdom

Background: Segmenting multiple myeloma (MM) into subgroups with distinct pathogenesis and clinical behavior is critical to implement a targeted therapy approach and improve prognosis for patients. Current technologies have elucidated major translocation groups and recurrent copy number changes with varying effects on prognosis. However, minor translocation and mutational groups remain poorly described due to limited sample numbers and small datasets. The availability of multiple sets of high quality genomic data associated with clinical information, cytogenetics, and outcomes provides an opportunity to create an integrative genomic predictor using mutational, chromosomal, and gene expression alterations to develop a classification system to segment MM into therapeutically meaningful subgroups.
Aims: The Multiple Myeloma Genome Project (MGP) is a global collaborative research initiative that aims to develop a molecular segmentation strategy for MM to inform development and deployment of clinically relevant tests that could improve diagnosis, prognosis, and treatment of patients with MM.
Methods: We have established a dataset representing 1766 MM patients for which whole exome sequencing (WES; n=1367), Whole Genome Sequencing (WGS; n=779), and expression data from RNA-Seq and Expression arrays (n=1059) were available. Data were derived from the Myeloma XI trial, Dana-Faber Cancer Institute/Intergroupe Francophone du Myeloma, The UAMS Myeloma Institute and the Multiple Myeloma Research Foundation (IA1 – IA9). Data were investigated for genetic abnormalities following preprocessing with state of the art methods and algorithms.
Results: Our analysis is focused on data from newly-diagnosed MM patients (n=1751), which is the majority of our dataset. We have begun to integrate genomic dataset with various correlates. Based on our data, we have at least