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ORAL ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Harnessing Btki Therapy By CDK4/6i Control of T Effector Memory Cells and T Cell Surveillance in Mantle Cell Lymphoma

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Drug resistance remains a formidable challenge in mantle cell lymphoma (MCL). Cell cycle dysregulation driven by aberrant Cyclin D1 and CDK4 expression is a hallmark for MCL, providing a rationale for targeting the cell cycle in MCL therapy. We have demonstrated in preclinical studies that inhibition of CDK4/6 not only induces early G1 cell cycle arrest, but also reprograms MCL cells for cytotoxic killing. On this basis, we investigated if inhibition of cyclin D1/CDK4 with palbociclib (CDK4/6 inhibitor) could reprogram recurrent MCL to deepen and prolong the ibrutinib (BTK inhibitor) response in a phase I clinical trial, and the resistance mechanism by longitudinal genomic analysis of sequential samples from individual patients in the context of the clinical response.

Palbociclib was administered to MCL patients on days 1-21 of a 28-day cycle; ibrutinib was given continuously. For longitudinal genomic analysis, sequential tissue and blood specimens from 27 evaluable MCL patients before and during therapy, and on progression were collected. Single cell RNA-seq (scRNA-seq) of PBMC or the monocytic fractions from bone marrow and lymph node (53 samples) was performed using a unique in house MCL cell-specific library. PBMC from normal donors (n=4) and untreated MCL patients (n=4) served as controls. The data were then subject to multiplex analysis with whole transcriptome sequencing and whole exome sequencing of MCL cells isolated from the same specimens, as well as flowcytometry in conjunction with IHC. Total numbers of MCL and immune cells in each specimen were deduced by integrating complete blood counts (CBC) with differential with scRNA-seq or flowcytometry.

CDK4/6 Inhibition appears to deepen and prolong the BTKi response, with a CR rate of 42% and 5 patients (2 CR and 3 PR) remained on therapy for ~ 9 years. Longitudinal scRNA-seq (210,000 cells) revealed that MCL cells comprise 4 major transcriptomically distinct clusters. Cluster 1 (C1) resembles quiescent normal B cells; C2 mimics hyper-activated B cells that are enriched for signatures of BCR and cytokine signaling; C3 represents non-proliferating, long-lived MCL cells; and C4 is highly proliferative. RNA velocity analysis further indicates that having progressed to late G1 after evading CDK4/6 inhibition, MCL cells in C2 are in transit to C4. By contrast, C3 is a terminal node of MCL cells arrested in late G1 with elevated BCL2 expression

Primary resistance and progression on therapy were associated with a marked expansion of either long-live non-proliferating C3 MCL cells, or C2 MCL cells that fuel the proliferating C4 MCL cells. Resistance was also associated with a profound reduction in MHC I and MHC II expression on MCL cells in some patients. Moreover, concurrent with disease progression, CD8+ T and CD+T cells were rapidly depleted by different mechanisms (***Figure 1***). CD8+ T cells were proliferating and eliminated by exhaustion, as evidenced by the marked expression of *LAG3 TIGIT* and *TIM3*, and a failure to maintain CD8+ T effector memory cells. By contrast, CD4+ T cells were not proliferating and appeared die from glucose starvation. Collectively, these data suggest that T cell maintenance and surveillance is pivotal in sustaining a durable clinical response to combined inhibition of CDK4/6 and BTK.

Guided by these discoveries, we have now restored BTKi sensitivity *ex vivo* in MCL cells from resistant patientsby targeting patient-specific expansion of C2 or C3 *in vivo*. In one resistant patient, this led to the maintenance of CD4+ and CD8+ T effector memory cells and a CR in response to venetoclax plus BTKi for nearly 3 years and continuing.

In summary, by integrated longitudinal scRNA-seq analysis of a hypothesis-driven therapy, we have provided the first evidence that 1) MCL cells comprise 4 major transcriptomically distinct clusters; 2) resistance to CDK4/6i is associated with expansion of C2 that fuels the proliferating C4 MCL cells, or the long-lived C3 MCL cells with elevated BCL2 expression; 3) CDK4/6i harnesses BTKi response by promoting the maintenance of effector memory T cells; 4) combined inhibition of BCL2 and BTK overrides resistance to CDK4/6i and BTKi, leading to a durable clinical response; and 5) T cell surveillance is pivotal for a durable response to CDK4/6, BTK or BCL2 inhibition; implicating genome-guided combination therapy to overcome therapy resistance in MCL.

Disclosures Maddocks: *AbbVie:* Consultancy; *AstraZeneca:* Consultancy, Research Funding; *BMS:* Consultancy, Research Funding; *Celgene:* Consultancy, Research Funding; *ADC Therapeutics:* Consultancy; *Incyte:* Consultancy, Honoraria; *Genentech:* Consultancy; *GenMab:* Consultancy; *Janssen:* Consultancy, Honoraria; *Morphosys:* Consultancy; *Pharmacyclics:* Consultancy, Research Funding; *Gilead/Kite:* Consultancy; *BeiGene:* Consultancy; *Epizyme:* Consultancy; *Eli Lilly and Company:* Consultancy; *Seattle Genetics:* Consultancy; *Novartis:* Research Funding; *Merck:* Research Funding. **Blum:** *Seattle Genetics:* Research Funding; *BMS:* Research Funding; *Cullinan Oncology, Inc.:* Research Funding. **Ruan:** *AstraZeneca:* Consultancy, Research Funding; *BMS:* Research Funding; *Genentech:* Research Funding; *Daiichi Sankyo:* Research Funding; *Secura Bio:* Consultancy. **Leonard:** *AbbVie, AstraZeneca, Astellas, Bayer, BeiGene, BMS, Calithera, Constellation, Eisai, Epizyme, GenMab, Grail, Incyte, Janssen, Karyopharm, Lilly, Merck, Mustang Bio, Pfizer, Roche/Genentech, Seagen, Second Genome, Sutro:* Consultancy; *National Cancer Institute, Leukemia and Lymphoma Society, Genentech, Epizyme, Janssen:* Research Funding. **Bartlett:** *ADC Therapeutics, Autolus, BMS/Celgene, Forty Seven, Gilead/Kite Pharma, Janssen, Merck, Millennium, Pharmacyclics, F. Hoffmann-La Roche Ltd / Genentech, Inc., Seattle Genetics:* Research Funding; *ADC Therapeutics, Foresight Diagnostics, Kite, F. Hoffmann-La Roche Ltd / Genentech, Inc., Seattle Genetics:* Membership on an entity's Board of Directors or advisory committees; *Washington University School of Medicine:* Current Employment. **Martin:** *AbbVie, AstraZeneca, Beigene, Epizyme, Genentech, Gilead, Janssen, Pepromene, Daiichi Sankyo:* Consultancy.

OffLabel Disclosure: Palbociclib is a CDK4/6 inhibitor FDA-approved for breat cancer treatment. It was used off-label in combination with ibrutinib in a phase I clinical trial in patients with relapsed/refractory mantle cell lymphoma.

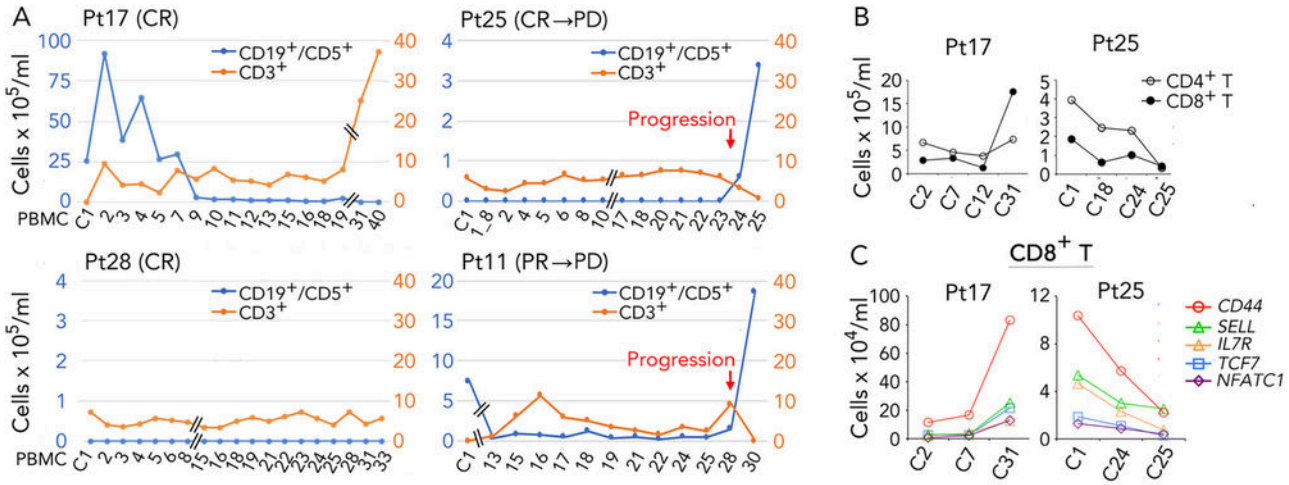

Figure 1

A, Absolute numbers of MCL cells (CD19+/CD5+, blue y-axis on the left) and CD3 T cells (Orange y-axis on the right) per ml of blood collected on the first day of indicated treatment cycle (C) were deduced by integrating FACS analysis with CBC/differential. Pt17 (CR); Pt28 (CR) and Pt25 (CR→PD) both had tumor cells in the lymph nodes but not in circulation initially; Pt11 (PR→PD). B, Total numbers of CD4+T and CD8+ T cells per ml of blood at indicated cycle of treatment in Pt17 and Pt25, deduced by multiplex analysis of absolute T cell numbers in A with scRNA-seq analysis. C, Longitudinal analysis of total number of CD8+ T effector memory cells in Pt17 and Pt25, based on gene expression in scRNA-seq analysis.

Figure 1

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