Targeting the Cell Cycle in Multiple Myeloma

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Goals
To inhibit tumor cell proliferation
To sensitize them for cytotoxic killing
To advance rational design of combination therapy

Success in targeting the cell cycle in cancer with broad-Spectrum CDK inhibitors has been modest due to a lack of specificity and high toxicity.
The Cell Cycle

CDK: Cyclin-Dependent Kinase
p18\textsuperscript{INK4c} (CDKN2C)
**p18^{INK4c}** is required for inhibition of CDK4/6 in both B cell activation and terminal differentiation.

CDK4/CDK6-specific phosphorylation of Rb and cell proliferation increase with disease progression in MM

Coordinated dysregulation of CDK4-cyclin D1 or CDK4/6-cyclin D2 precedes proliferation in MM

Targeting CDK4/6 with PD 0332991

- Only known selective CDK4/CDK6 inhibitor (IC$_{50}$ 11 nM)
- Induces early G1 arrest
- Reversible
- Orally bioavailable pyridopyrimidine
- Low in toxicity

- Selectively and potently inhibits CDK4/6 phosphorylation of Rb in primary human myeloma cells (IC$_{50}$ 60 nM)

- Inhibits tumor growth in the NOD-SCID human myeloma xenograft models and the immune-competent mouse 5T models

Fry et al., 2004, Mol Cancer Ther
Baughn et al., 2006, Cancer Research
Menu et al., 2008, Cancer Research
Hypothesis

- Prolonged inhibition of CDK4/6
- Halting gene expression in early G1
- Forcing an imbalance in gene expression
- Sensitizing tumor cells to cytotoxic killing
Hypothesis

Release of prolonged early G1 block

Cell cycle synchronization
incomplete restoration of scheduled gene expression

Further sensitizing tumor cells to cytotoxic killing
PD inhibition of CDK4/6

- early G1 arrest
- synchronous cell cycle progression upon release of the G1 block

Huang and Di Liberto
Prolonged early G1 arrest (pG1) sensitizes MM cells to cytotoxic killing, greater in S phase synchronization and proportional to the time of G1 arrest.
Prolonged inhibition of CDK4/6

Expresses only genes programmed in early G1

Forcing an imbalance in gene expression

Release of early G1 block

Cell cycle synchronization

Incomplete restoration of scheduled gene expression
Phase I/II clinical trial of PD 0332991-bortezomib-dexamethasone combination therapy in MM

Schedule B

Cyclin D + CDK4/6

PD 0332991

Bortezomib + Dexamethasone

Cycles 1-10

D1  D8  D11  D15  D18  D21

Go

M

G2

G1

S

Bz+Dex
Phase I/II PD 0332991 sequential combination therapy clinical trial in relapsed refractory MM

• First clinical study to target CDK4/6 in sequential combination therapy in cancer

• PD preferentially and completely inhibits CDK4/6 in MM patients (n=16)

• Inhibition of the cell cycle by PD in vivo is reversible

• PD is generally well tolerated, with neutropenia as the most common adverse event

• 1 VGPR, 1 PR and 3 stable disease in these heavily pretreated myeloma patients

Phase II in progress (Ruben Niesvizky, Tomar Mark)
PD 0332991 potently inhibits tumor cell proliferation by preferential inhibition of CDK4 and CDK6 in MM

Red: IRF4: MM
Blue: pSRb or
Blue: Ki67

Ely, DiLiberto, Huang, Niesvizky
Hypothesis

Prolonged inhibition of CDK4/6
<<>
Halting gene expression in early G1
Forcing an imbalance in gene expression
Sensitizing tumor cells to cytotoxic killing
Inhibition of CDK4/CDK6

- Early G1 genes
- Late G2, S, G2/M genes

Huang, Di Liberto et al, Blood, 2012
Prolonged inhibition of CDK4/6

*Expresses only genes programmed in early G1*

*Forcing an imbalance in gene expression*

Release of early G1 block

*Cell cycle synchronization*

*Incomplete restoration of scheduled gene expression*
Does prolonged early G1 arrest (pG1) induced by CDK4/CDK6 inhibition sensitizes myeloma cells to IMiD killing?

- Enhances IMiD clinical efficiency at lower doses
- Mechanism of IMiD killing
- Biomarker for IMiD killing
- Maintenance therapy
- Control of MM stem cell renewal
- Control of secondary malignancy
pG1 enhances and accelerates lenalidomide killing of primary myeloma cells in stromal co-culture
pG1 sensitizes primary myeloma cells to IMiD killing independent of prior treatments or cycling status, but dependent on Rb

Lenalidomide: 17/21        Pomalidomide: 3/4
pG1 sensitizes myeloma cells to IMiD-mediated apoptosis through synergistic increase in CRBN and loss of IRF4 mRNA and protein

IRF4: essential MM survival factor
Cereblon (CRBN): E3 ligase mediate IRF protein degradation
Inhibition of CDK4/CDK6

Early G1 genes

Late G2, S, G2/M genes

Huang, Di Liberto et al, Blood, 2012
Whole Transcriptome (RNA)-sequencing

RNA abundance, variant, indel

50x50 paired-end RNA sequencing on a HiSeq2000, 76 million reads per sample

Use the Burrow-Wheeler Aligner to align reads to the genome (Building 37)

SAMtools and Genome Analysis Toolkit to call non-reference variants.

D. Chiron, X. Huang, M. DiLibiberto, C. Mason
Analysis of RNA-sequencing (RNA-Seq) Data
**Genetic variation:** (SNVs, indels, CNVs)

**Algorithms:** Rmake, BWA/SAMtools

**Counts and differential expression by gene, exon, allele, splice isoform, & transcript**

Algorithms: TopHat, Rmake, Cufflinks, BayesASE

**Predict gene fusions, polyA sites**

Algorithms: Rmake, Alexa, SnowShoes

**Find ncRNAs and new TARs**

Algorithms: Rmake, Aceview

**Genetic variation:** (SNVs, indels, CNVs)

Algorithms: Rmake, BWA/SAMtools

GATK, HGVS, VarScan
High reproducibility with low input

A

10ng

100ng

1000ng

B

Raw Reads per Gene (10ng)

R²=0.95

Genomics and Bioinformatics
Analysis of Whole Exome Sequencing (WES) Data
Multi-threaded Genetic Analysis for WES

**Tools Used:**

- **Bwa** (PMID: 20080505)
- **Bowtie** (PMID: 19261174)
- **Samtools** (PMID: 19505943)
- **GATK** (PMID: 20644199, PMID: 2147881)
- **Dindel** (PMID: 20980555)
- **Picard**
- **Mosaik**
- **Vcftools**

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**BWA**

1. **HumanRef 1kG_v37**
2. **HumanRef HG19**
3. **HuRef idx**
4. Indexed HuRef (color/base space)
5. Gapped global Alignment with BWA and local SW
6. Raw sequence files
7. Compressed FASTA to save disk space

**Raw sequence files**

- sai
- Sam/bam
- Coordinate sort
- Mark & remove PCR duplicates
- Realign intervals for Indel calling
- Identify regions where alignment artifacts may be present and correct them
- Remove optical and PCR duplicates
- Q>20
- Intersect with Capture region
- Select only reads within the Target Genome
- FixMate in PE
- Ensure the paired end reads are properly paired
- BaseQ recalibration
- Recalibrate the quality-score based on instrument, read and base parameters

**Variant databases**

- dbSNP, COSMIC, NCBI gene, OMIM, Hapmap 1kG, mirBASE, TCGA

**Calling SNVs**

- Call SNVs
- Call Indels, VCF4 file

**Vcftools**

- Automated Primer Design

**Variant databases**

- Annotating programs
- SeattleSeq
- CHARITYannotator: cBio Core

**Variant databases**

- Recurrence / Segregation

**Variant databases**

- Automated Primer Design

**Variant databases**

- Sanger Sequencing of Sequenom validation
Tumor-specific discovery pipeline

Alignments (BWA, X1:X0 filter)

Tumor bam file

Normal bam file

Analysis in ICB Cluster

algorithm: samtools, GATK, Bkdancr
output: SNVs, Indels, SVs

Tumor variant file

Normal variant file

Filter for known variant

Variant Annotation

Tumor specific

Normal

Both/germline

Compare to other patients in ICGC/TCGA/dbGAP data

Common/Rare

SNVs, Indels, SVs

Emerging/Evolved

Genomics and Bioinformatics
Integrative Mutational Profiling
Discovery of a novel mechanism for IMiD killing of primary myeloma cells and sensitization by pG1 through integrative WTS and WES analysis
pG1 enhances induction of type 1 interferon and TRAIL by lenalidomide...
This is mediated by derepression of IRF7 through loss of IRF4
Does induction of prolonged early G1 arrest (pG1) by CDK4/CDK6 inhibition sensitizes myeloma cells to IMiD killing? -YES!

✓ Enhances IMiD clinical efficiency at lower doses
✓ Mechanism of IMiD killing—two novel pathways
  • Biomarker for IMiD killing
    RNA-sequencing of serial biopsies from clinical trials
    Whole genome sequencing before and after treatment
    High throughput shRNA screen, functional validation
  • Maintenance therapy
  • Control of MM stem cell renewal—FoxO and inhibition of CDK4/6
  • Control of secondary malignancy—FoxO (AML)
Does pG1 sensitizes lymphoma cells To cytotoxic killing?
Mantle Cell Lymphoma (MCL)

Non-Hodgkin’s lymphoma with an overall poor prognosis.

Incurable due to the eventual development of drug resistance

Constitutive cyclin D1 expression due to chromosomal t(11;14) translocation and mutations

Overexpression of CDK4
Targeting CDK4/CDK6 in MCL

Single agent PD 0332991 study
—Testing pG1 (prolonged inhibition of CDK4/CDK6)

• Inhibition of CDK4/CDK6 by PD 0332991 leads to prolonged G1 arrest (pG1) and increased tumor-specific cell death in MCL (n=17)

• PD (125 mg/d orally 21 of 28 d) is generally well tolerated with neutropenia, fatigue and diarrhea as most common adverse events

• 1 complete response, 2 partial response, 5 SD > 1 year

Leonard, Vallabhajosula, Martin, Chen-Kiang et al, Blood 2012
Phase I study of PD + bortezomib in patients with recurrent MCL (P. Martin)

3+3 design

Biopsy ★ ★ ★ ★
Identification of novel molecular targets and candidate Biomarkers by RNA-sequencing analysis of primary tumor cells from serial biopsies of clinical trials
Summary-
• Selective inhibition of CDK4/CDK6 with PD 0332991 leads to prolonged early G1 arrest (pG1) and cell cycle synchronization in all MM and MCL tumors.
• Cell cycle control by inhibition of CDK4/CDK6 alone is insufficient to predict clinical response in MM or MCL.

Question-
• What are the genes that mediate cell cycle control of differential clinical response?

Strategy-
• RNA-Seq of serial biopsies from the clinical study
• Sensitizing lethal suppression genome-scale shRNA screen
Inhibition of CDK4/6 is sufficient to control the cell cycle in MCL tumors in clinical trial –RNA-Seq analysis of serial biopsies
Inhibition of CDK4/CDK6

→ Early G1 genes

→ Late G2, S, G2/M genes

Huang, Di Liberto et al, Blood, 2012
Selective genes are differentially expressed in MCL tumors in prolonged early G1 arrest in the context of clinical response.

RNA-Seq analysis of transcript abundance on d8 (pG1) vs d0

Chiron, Martin, Leonard, Mason, unpublished
Multiple Myeloma

RNA-Seq analysis of transcript abundance on d8 (pG1) vs d0
RNA-Seq analysis of transcript abundance on d8 (pG1) vs d0

Chiron, Huang, Mason, unpublished
7 genes are oppositely regulated in prolonged early G1 arrest induced by CDK4/6 inhibition in tumor cells of responders vs non-responders in both MM and MCL

3 genes are transcription factors
   —Metabolism, amino acid transport
   ---Glutathione –ROS

2 genes are cell surface receptors

2 genes are involved in cell migration and division
Targeting CDK4/CDK6 in hematologic malignancies

Significance

• Both inhibits the cell cycle in tumor cells and sensitizes them to cytotoxic killing forcing an imbalance in gene expression by induction of prolonged early G1 arrest (pG1)

• Mechanism-based combination therapy
  
  pG1  iMiDs, GS-1101, ibrutinib
  pG1-S  bortezomib, carfilzomib, Ara C
Targeting CDK4/CDK6 in hematologic malignancies (continued)

**Significance**

- Sensitizing genome-scale pooled shRNA screen
  
  *cell cycle sensitizing genes*

- WTS of serial biopsies from clinical trials of CDK4/CDK6-based combination therapy in MCL, MMC and soon in AML.

*Molecular biomarkers and targets*

*Genome-based patient selection and therapy stratification*
Targeting CDK4/CDK6 in combination therapy

**Partner agent (low dose, selective)**

- **PD 0332991**

- **Weill-Cornell**
  - **Mantle cell lymphoma**: Phase I single agent study
  - **Multiple Myeloma**: Phase I/II PD-bortezomib-Dex
  - **Mantle cell lymphoma**: Phase I PD-bortezomib (ongoing)
  - **Acute myeloid Leukemia**: Phase I PD-AraC (2/2013)
  - **Mantle cell lymphoma**: Phase I PD-Ibrutinib (2013)
  - **Multiple myeloma**: Phase I PD-Lenalidomide (2013)

- **Advanced Solid tumors**: Phase I single agent (2006, completed)
- **Breast cancer**: Phase I/II front line combination therapy (2009-)
- **Metastatic liposarcoma**: Phase I single agent (9/2010--)
- **Glioblastoma**: Phase I single agent (10/2010--)
- **Non-small cell lung carcinoma**: Phase I single agent (2/2011--)
The Team

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