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Identification and characterization of mutations in multiple myeloma

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Background & Aim of the Study

Multiple myeloma (MM) is a largely incurable hematological malignancy characterized by the accumulation of monoclonal plasma cells in the bone marrow, hematopoietic insufficiency, bone lesions and hypercalcemia. Additionally MM shows a broad spectrum of genetic heterogeneity with different clonal subtypes. In a previous whole-exome study we found an enrichment of somatic mutations in adhesion molecules, receptor-tyrosine kinases (RTK) and their downstream effectors predicting an inter- and intra- individual pathway redundancy.

The aim of the current study was to further elucidate the role of RTK in MM. The coding DNA sequence of the RTKs EGFR, ERBB3, IGF1R, NTRK1 and NTRK2 of 75 uniquely treated MM patients of the DSMM and 12 MM cell lines was sequenced using an amplicon sequencing approach.

Previous Findings

Definition of a Protein Interacting Network (RTK-signaling, adhesion molecules (n=61))

→ gene affected in at least one primary MM (n=43) and mutation „damaging“

95% of primary MM:
at least 1 mutation
=
Inter-individual pathway redundancy

~50% of primary MM:
at least 2 mutations
=
Intra-individual pathway redundancy

Methods

Screening for mutations in *RTKs* in
75 MM patients of the DSMM + 12 cell lines

Library Preparation
Access Array System

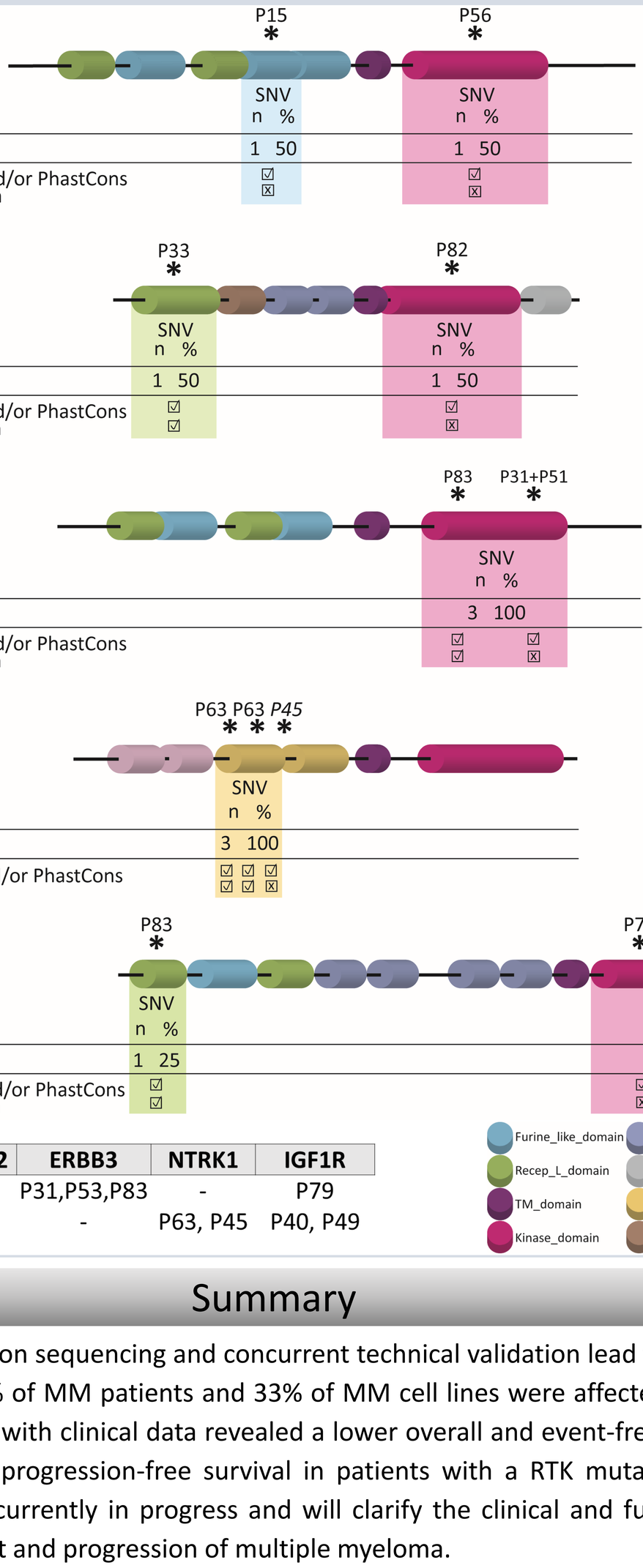
dsmm deutsche studiengruppe multiples myelom

Sequencing, MiSeq (Illumina)

Technical validation + Correlation

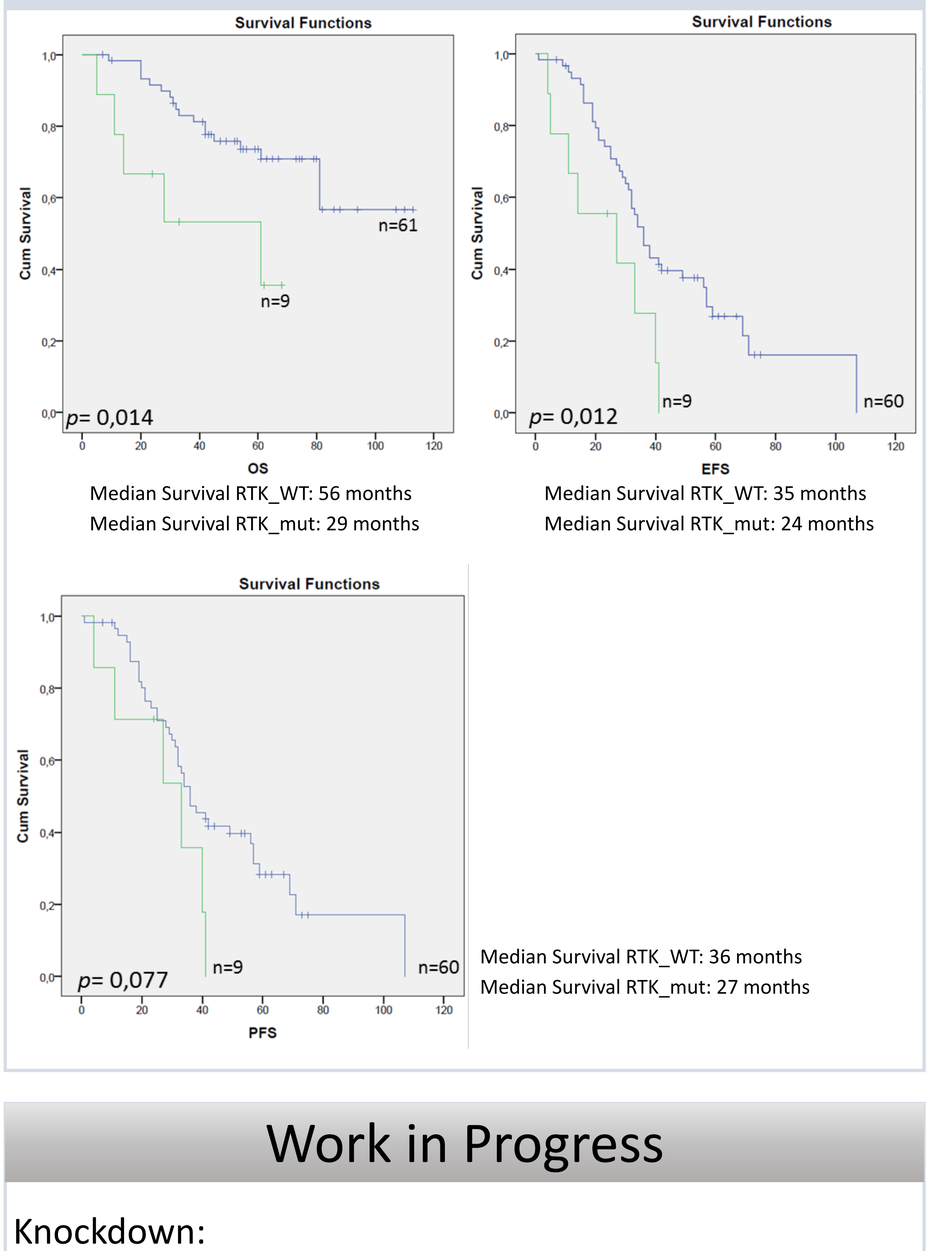
Results

Frequency of mutations and affected regions in receptor-tyrosine kinases



SNV	EGFR	EPHA2	ERBB3	NTRK1	IGF1R
T + N	P15	P82	P31,P53,P83	-	P79
T (N n.a.)	P56	P33	-	P63, P45	P40, P49

Correlation with clinical data



Median Survival RTK_WT: 56 months
Median Survival RTK_mut: 29 months

Median Survival RTK_WT: 35 months
Median Survival RTK_mut: 24 months

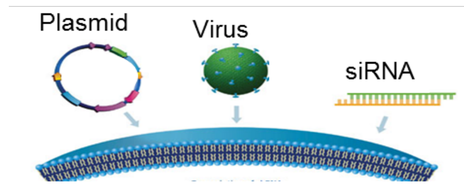
Median Survival RTK_WT: 36 months
Median Survival RTK_mut: 27 months

Work in Progress

Knockdown:
- siRNA and electroporation

Overexpression:
- ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kit

Kinase activity assay of RTKs with different mutations



Summary

In summary , the amplicon sequencing and concurrent technical validation lead to the discovery of novel mutations. 16% of MM patients and 33% of MM cell lines were affected by mutations in RTKs. The correlation with clinical data revealed a lower overall and event-free survival and a trend towards a lower progression-free survival in patients with a RTK mutation. Additional functional analysis are currently in progress and will clarify the clinical and functional role of RTKs in the development and progression of multiple myeloma.

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