Charles University
Faculty of Medicine in Hradec Králové

Unfavourable Biological Prognostic Factors in Multiple Myeloma

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Abstract of the Dissertation
Doctoral Study Programme: Internal Medicine

Hradec Králové

2019

Dissertation thesis was written during the combined doctoral study (Ph.D.) programme in Internal Medicine at the 4th Department of Internal Medicine - Haematology, Faculty of Medicine in Hradec Králové, Charles University.

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The defence will take place in Faculty Hospital Hradec Kralove, building no. 23, 3rd floor, lecture hall no 3.212 on September 26,2019 at 12:30pm.

This work has been supported by Mobility fund in year 2011.

The dissertation is available for inspection at the Study Department of the Dean´s Office, Faculty of Medicine in Hradec Králové, Charles University, Šimkova 870, 500 03 Hradec Králové (phone 495 816 134).

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Summary

Title I: Aurora kinase and FGFR3 inhibition results in significant apoptosis in molecular subgroups of multiple myeloma

In our pre-clinical study we examined the role of Aurora kinase and FGFR3 inhibition in MM using a small molecule inhibitor A1014907 which induced aneuploidy in MM cell lines at low nanomolar doses. However, A1014907 induced more pronounced and dose dependent apoptosis in cell lines with t(4;14) translocation. Translocation t(4;14) is observed in about 15% of patients with MM leading to constitutive activation of FGFR3 in two-thirds of these patients. Further investigation of the mechanism of action of A1014907 revealed potent FGFR3 pathway inhibition only in the sensitive cell lines. Thus, our results show that aurora kinase inhibition causes cell cycle arrest and aneuploidy with minimal apoptosis whereas inhibiting both aurora kinase and FGFR3 activity induced potent apoptosis in MM cells. This study evaluates the role of simultaneous inhibition of Aurora Kinases and FGFR3 pathway which are both important deregulated pathways in MM patients; inducing potent apoptosis.

Title II: Natural history of multiple myeloma with de novo del(17p)

Our clinical study involved comparing the outcomes of 310 newly diagnosed MM patients with del(17p) detected by FISH to patients with high-risk translocations (HRT) ($n = 79$) and standard-risk (SR) cytogenetics ($n = 541$). The median progression-free survival (PFS) following initial therapy for the three groups was 21.1, 22, and 30.1 months, respectively ($P = 0.437$- del(17p) vs. HRT); the median overall survival (OS) was 47.3, 79.1, and 109.8 months, respectively, ($P = 0.007$- del(17p) vs. HRT). PFS and OS for patients with relative loss of 17p ($n = 21$) were comparable to other patients with del(17p). The PFS was similar between the del(17p) and HRT groups when stratified for age, ISS stage or treatment. The OS of del(17p) and HRT groups were similar in presence of advanced age, ISS III stage or if patients did not receive a proteasome-inhibitor containing induction. ISS III stage, high LDH and HRT, but not the percentage of cells with del(17p) predicted shorter OS in patients with del(17p).

This study identified, factors predictive of OS in patients with de-novo MM and also found no difference in outcomes for patients with relative loss of del17p.

Title III: Impact of acquired del(17p) in multiple myeloma

We next studied the impact of acquiring del17p after the diagnosis of MM as the role of late acquisition of this deletion is not well described. The disease characteristics at diagnosis predicting for acquired del(17p) and its overall impact on patient survival is also not well studied.

We compared 76 patients with MM who were del(17p) negative at diagnosis, and acquired it later, and compared them to 152 control MM patients who did not acquire del(17p) at a comparable timepoint. Patients acquired del(17p) at a median of 35.6 months (range, 4.6-116.1) from diagnosis of MM, after a median of 2 (range, 1-10) lines of treatment. Patients with acquired del(17p) when compared to controls, had shorter median progression free survival (PFS) [23.0 vs. 30.1 months; P=0.032] and overall survival (OS) [68.2 vs. 106.1 months; P<0.001] from diagnosis.

The median PFS and OS after the detection of del(17p) were 5.4 months and 18.1 months respectively. High lactate dehydrogenase level [OR-3.69 (95% CI, 1.11-12.24)], and presence of t(4;14) [OR-2.66 (95% CI, 1.09-6.48)] or any high risk translocation [OR-2.23 (95% CI, 1.00-4.95)] at diagnosis predicted for acquisition of del(17p). High PC proliferative rate predicted shorter OS from detection of del(17p) [hazard ratio - 2.28 (95% CI, 1.31-3.96) (P=0.004).

Our study shows that acquisition of del(17p) is an important molecular event associated with reduction in OS in MM. Certain baseline factors may predict acquisition of del(17p). This needs validation in prospective datasets.

Background

Multiple Myeloma (MM)

MM is a monoclonal plasma cell(PC) proliferation disorder characterised by aaccumulation of terminally differentiated mature B cells which presents with disease-related features including anaemia, kidney insufficiency, bone lesions and hypercalcemia[1].

It is the 14th most common malignancy and the second most common hematological malignancy that accounts for 1 to 2% of all cancers in the United States and has an annual incidence of approximately 4-5 per 100,000 in the developed world and the mortality of 4.1/100,000/year [2, 3]. It is more common in the population over 60 years of age with about 10 % population under 50 and 2 % under 40 years at diagnosis. The median age at diagnosis is 69, and the median age at death is 75. The estimated new cases in the United States were 30,770 in 2018 with approximately 12,700 deaths secondary to the disease accounting for 2.1 percent of all cancer-related mortality. Encouragingly the death rates were falling by 0.5% each year between 2006-2015 with 5-year relative survival percent at 53 in 2015 compared to 26.3 % in 1975[2]. The prevalence of MM IN US was estimated at 124,733 in 2015[2].

Treatment options have significantly improved for patients with MM. Newer drug combinations have improved overall survival(OS) for these patients. It is still an incurable disease with likely disease relapse for most patients. Better treatment options need to be explored to minimize toxicity, improve tolerability and ideally normalize lifespan of the patients to non-affected population. This would need better understanding of the disease biology and treatment alternatives.

Chromosomal abnormalities

Broadly, molecular cytogenetic aberrations are classified as primary and secondary chromosomal abnormalities. This classification considers presence of primary chromosomal abnormalities in MGUS disease stage. Usually, primary chromosomal abnormalities are either trisomies or translocations. Trisomies usually involve odd number chromosomes including 3, 5, 7, 9, 11, 13, and 15 forming an aneuploid / hyperdiploid karyotype. Primary chromosomal translocations involve locus for heavy chain immunoglobulin IGH On chromosome 14 along with associated chromosome partners which most frequently are located on chromosome 4, 6,11,16 and 20[6]. Other less frequent Primary chromosomal abnormalities involve IgH translocations involve unusual partner chromosomes and simultaneous trisomies with IgH translocations [6].

FISH abnormality	**Approximate frequency (%)**	**Chromosome affected**
Trisomy(No IgH abnormality)	42	Odd number chromosomes
IgH translocations without trisomies	30	
t(11;14)	15	*CCND1(Cyclin D1)*
t(4;14)	6	*FGFR3 and MMSET*
t(14;16)	4	*C-MAF*
t(14;20)	<1	*MAFB*
Other IgH translocations with uncommon partner chromosomes	5	
IgH translocations with trisomies	15	
t(11;14)	3	*CCND1(Cyclin D1)*
t(4;14)	4	*FGFR3 and MMSET*
t(14;16)	1	*C-MAF*

t(6;14) Other IgH translocations with uncommon partner chromosomes	<1 7	*CCND3(Cyclin D3)*
Monosomy 14 without IgH translocations or trisomies	4.5	
Other cytogenetic abnormalities in the absence of IgH translocations or trisomy(ies) or monosomy 14	5.5	
Normal	3	

Table 1: Cytogenetic abnormalities in MM [7, 8]

Important high risk cytogenetic aberrations

T(4;14)(9p16;q32)

t(4:14) is the second most common IGH translocation seen in 10 to 15% of MM patients and about 25% of MM cell lines representing breakpoints on chromosome 4 within the proximity of FGFR3 and MMSET exon 5[9,10]. It is also noted to be less frequent in MGUS versus SMM and MM with a higher incidence in the patient less than 66 years of age[11].

T(4;14) is undetectable using conventional cytogenetics(g banding) all spectral karyotyping due to the telomeric location of translocation and is detected by reverse transcriptase polymerase chain reaction(RT-PCR) or iFISH[12]. Interestingly, 30% of patients with t(4:14) lack FGFR3 expression. However, this patient population maintains a poor prognosis highlighting that likely dysregulation of both the genes is implicated in the poor overall survival[10, 13].

Study	% t(4;14), (N= total patients)	PFS t(4;14) - months	PFS all patients-months	OS t(4;14)-months	OS all patients-months	Treatment
Chang et al.[14]	15 (120)	9.9	25.8	18.3	48.1	HDT and ASCT
Gertz et al.[15]	26(153)	8.2	17.8	18.8	43.9	HDT and ASCT
Chang et al.[16]	6(40)	10.4	6.8	15.1	10.3	Bortezomib
San Miguel et al.[17]	4(682) +/- t(14;16)	19.8	21.7	Not reached (Low-risk group only)	Not reached (low-risk group only)	Bortezomib/ Melphalan/ Prednisolone
Reece et al.[18]	28 (102)	8.0 (at progression)	7.1 (at progression)	23.7	18.13	Len/Dex
Aver-Loiseau et al.[19]	14 (184)	5.5	10.6	9.4	15.4	Len/Dex
Chan H et al.[20]	75 (75)	33.5		69.6		HDT/ Chemo alone

Table 2: Highlights some important studies evaluating outcomes of patients with t(4;14). Adapted from Kalff et al.[21]

Patients respond poorly to treatment as noted in above treatment with t(4;14). No specific targeted inhibitors have been successfully trialled in this patient cohort. Our study aimed at identifying the role of pFGFR3 inhibitor showed significant apoptosis in MM cell lines with t(4;14) and activated FGFR3 pathway.

Del17p/Monosomy 17

Loss of functional tumor suppressor gene p53 is an unfavorable high-risk genetic aberration in MM identified as deletion (del17p13.1/del17p) on FISH or monosomy 17 on karyotype [22]. These are considered subsequent events given increasing incidence with disease progression/relapse [23]. Mutations in the chromosomal region are rare (3%); however, increase with disease progression [24, 25]. Almost certainly, these mutations are associated with del17p portending an adverse OS [25-27]. Del17p13.1 is frequent and have been reported in about 11% of patients with newly diagnosed Multiple Myeloma [12, 28, 29]. MM patients with del17p have a poor prognostic outcome despite the use of newer therapeutic combinations [30].

P53(17p13.1) regulates major pathways of cellular homeostasis in stressed or damaged cells[31]. Its functional loss exists in more than 50 % of human cancer [31-34]. Functional loss of p53 in MM is associated with a poor disease outcome and frequent uncommon MM presentations including EMD and PCL [35-37]. Although some treatment response has been reported with Bortezomib based regimens[24, 25], patients with Del17p usually achieve unsatisfactory survival benefits to novel agent treatment and/or Stem cell transplantation (SCT), when compared to the MM patients without Del17p[30, 38-40].

Objectives

I) Identification of molecular target for aurora kinases and t(4;14) translocation with fgfr3 specificity in MM

- Simultaneous Aurora kinase and pFGFR3 inhibition by A1014907 produces potent cytotoxicity in t(4;14) MM cell lines
- The target specificity for pFGFR3 with Aurora kinase inhibition is further seen in CLL cell lines with activated pFGFR3 pathway opening possible targets in other malignancies as single agent or in combination

II) Study of natural history of 17p loss in MM:

IIa) De-novo

- Del17p at diagnosis is associated with poorer overall survival(OS) compared to high-risk translocation(HRT) and standard risk(SR) cytogenetics group
- High LDH, ISS III and HRT predict for shorter OS in patients with del17p patients

IIb) Acquired

- Acquisition of del(17p) by FISH is associated with reduced OS in patients with multiple myeloma
- High LDH and high-risk translocations at diagnosis predicted acquisition of del(17p) and high plasma cell proliferative rate predicted reduced overall survival from detection of del(17p).

Objective I - Methods and results

Cytotoxicity and anti-proliferative effects of A1014907 (Tables 3 & 4)

We first assessed the cytotoxic and anti-proliferative effects of A1014907 on MM cells. For this, we treated a panel of MM cell lines with various doses of A1014907 for 72 hrs and examined the cytotoxicity induced by the drug. We observed that cell lines OPM2, KMS11, KMS18, KMS28BM, H929, KAS6, LP1, OPM1 and KMS34 were significantly more sensitive to A1014907 treatment when compared to the other cell lines examined including MM1S, MM1R, U266, Dox40, RPMI8226, KP6, ANBL6 and ALMC1

t(4;14) translocation	**IC 50**	**Non t(4;14) Translocation**	**IC 50**
Kas6	10nM	MM1S	>500nM
KMS 11	10-50nM	MM1R	>500nM
KMS 18	500nM	DOX	> 1000nM
KMS 28	500nM	RPMI	> 1000nM
H929	10nM	U266	> 1000nM
OPM2	>500nM		

Table 3: Cytotoxic effects of A1014907 in MM cell lines

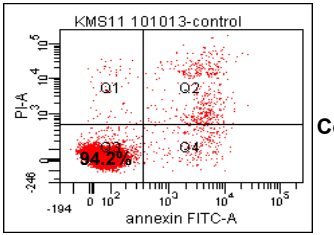
t(4;14) translocation	IC 50	Non t(4;14) Translocation	IC 50
Kas6	10nM	MM1S	> 500nM
KMS 11	10nM	MM1R	10 - 500nM
KMS 18	10 - 50nM	DOX	100nM
KMS 28	10 - 50nM	RPMI	> 500nM
H929	10nM	U266	50nM
OPM2	500nM		

Table 4: Anti-proliferative effects of A1014907 in MM cell lines

A1014907 induces dose and time dependent apoptosis in t(4;14) compared to non-t(4;14) - (Annexin/Pi staining) (Fig 1-2)

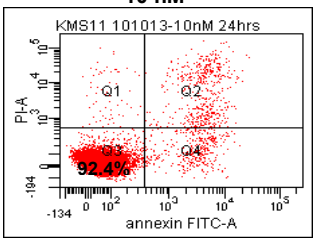
Given that A1014907 induced more potent cell death in cells with t(4;14) translocation than in cells lacking this translocation, we next performed assays to confirm if the cell death occurred through induction of apoptosis. We used representative cell lines from each group with KMS11[t(4;14)] and MM1S[non t(4;14)] cell lines and treated them with indicated concentrations of A1014907 for various time points. We observed that A1014907 induced potent apoptotic cell death in KMS11. However, MM1S cells showed resistance to A1014907 treatment with minimal increase in apoptosis.

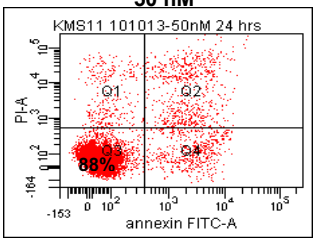
Fig 1: A101 causes dose and time dependent increased apoptosis in KMS 11 (Annexin/Pi)

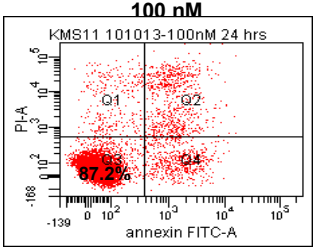


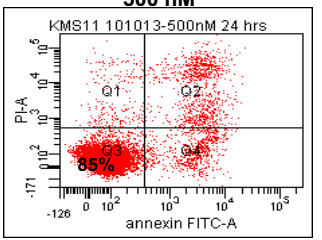
Control

10 nM | **50 nM** | **100 nM** | **500 nM**









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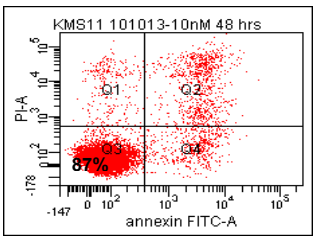

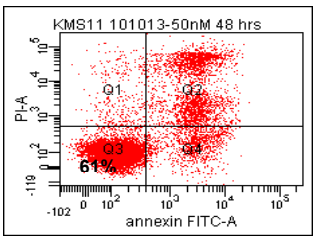

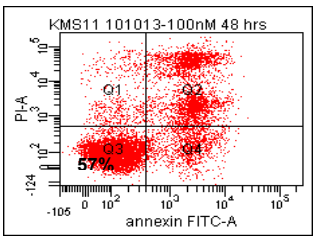

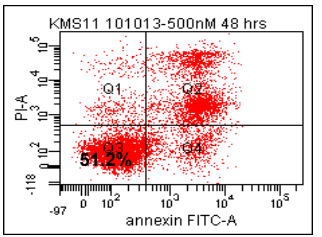

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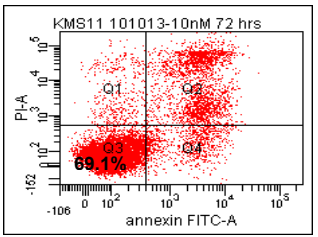
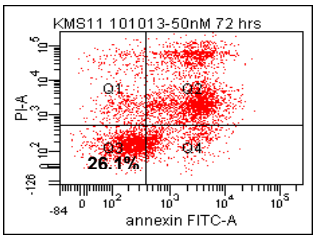
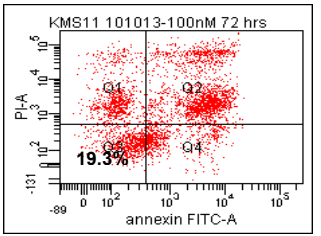
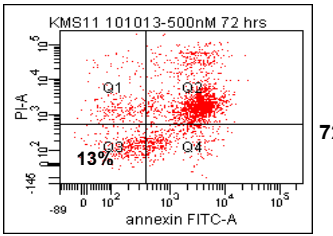
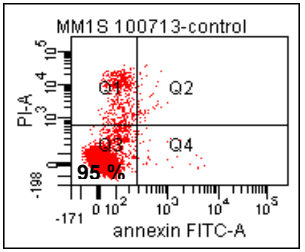








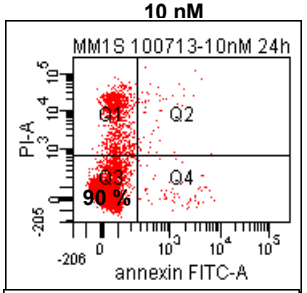
72hr

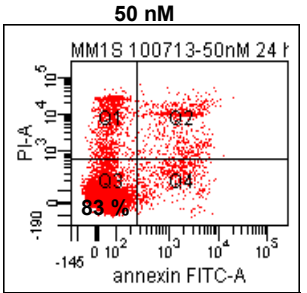


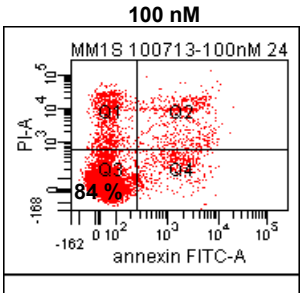
Control

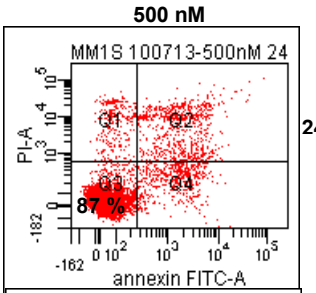
Fig 2: A101 causes limited dose and time dependent apoptosis in MM1S

10 nM | **50 nM** | **100 nM** | **500 nM**









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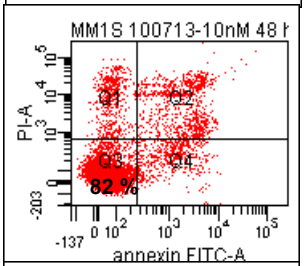

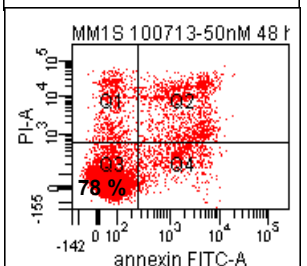

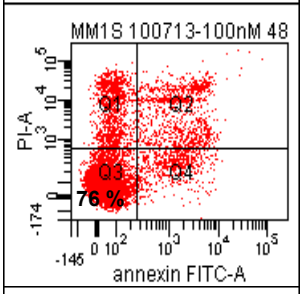

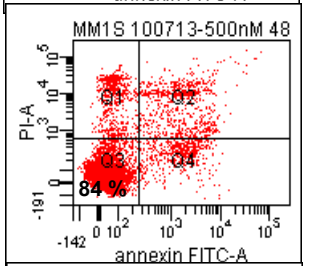

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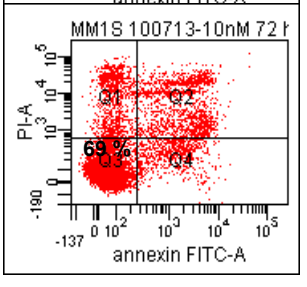
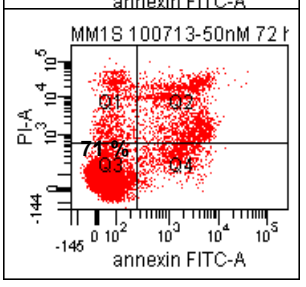
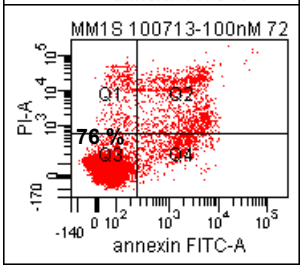
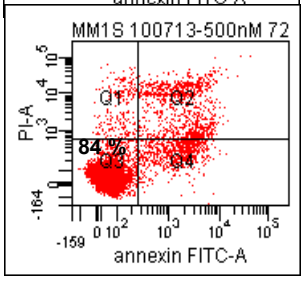








72hrs

A1014907 inhibits Aurora Kinases A and B and proteins involved in cell cycle machinery (Fig 3) and specifically pFGFR3 in t(4;14) cell lines(Fig 4a & b)

We next examined the mechanism of action of A1014907. We treated KMS18 and MM1S cells with indicated concentrations of A1014907 and examined the expression levels of aurora kinases and proteins involved in cell cycle progression. Down regulation of phospho Aurora A and phospho Histone H3, a substrate of Aurora B and a biomarker of mitosis in all the cell lines was noted (Fig 3). Surprisingly, we also observed that A1014907 caused down regulation of total Aurora A in both cell lines.

Our results so far suggest that A1014907 inhibits proliferation in all MM cell lines through the down regulation of Auroras A and B. However, A1014907 induced significant cell death only in MM cells with t(4;14) translocation which is associated with increased FGFR3 expression[41]. We therefore hypothesized that A1014907, in addition to being an aurora kinase inhibitor was also able to inhibit FGFR3 causing increased apoptosis in cells with t(4;14) translocation. We performed a receptor tyrosine kinase (RTK) array using KMS11 cells left untreated(fig 4a) or treated(fig 4b) with indicated dose of A1014907. The results showed down regulation of pFGFR3 by A1014907 (fig 18.b).

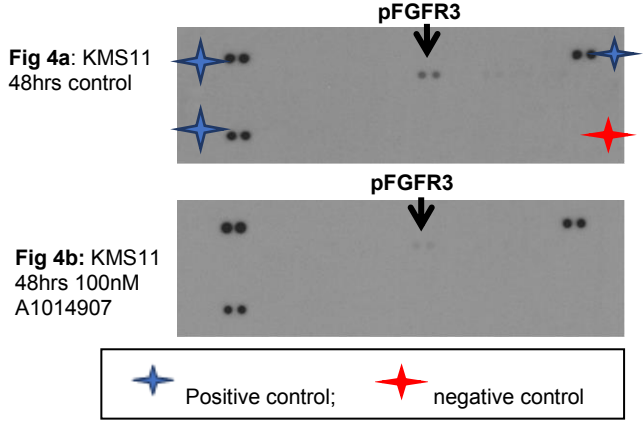
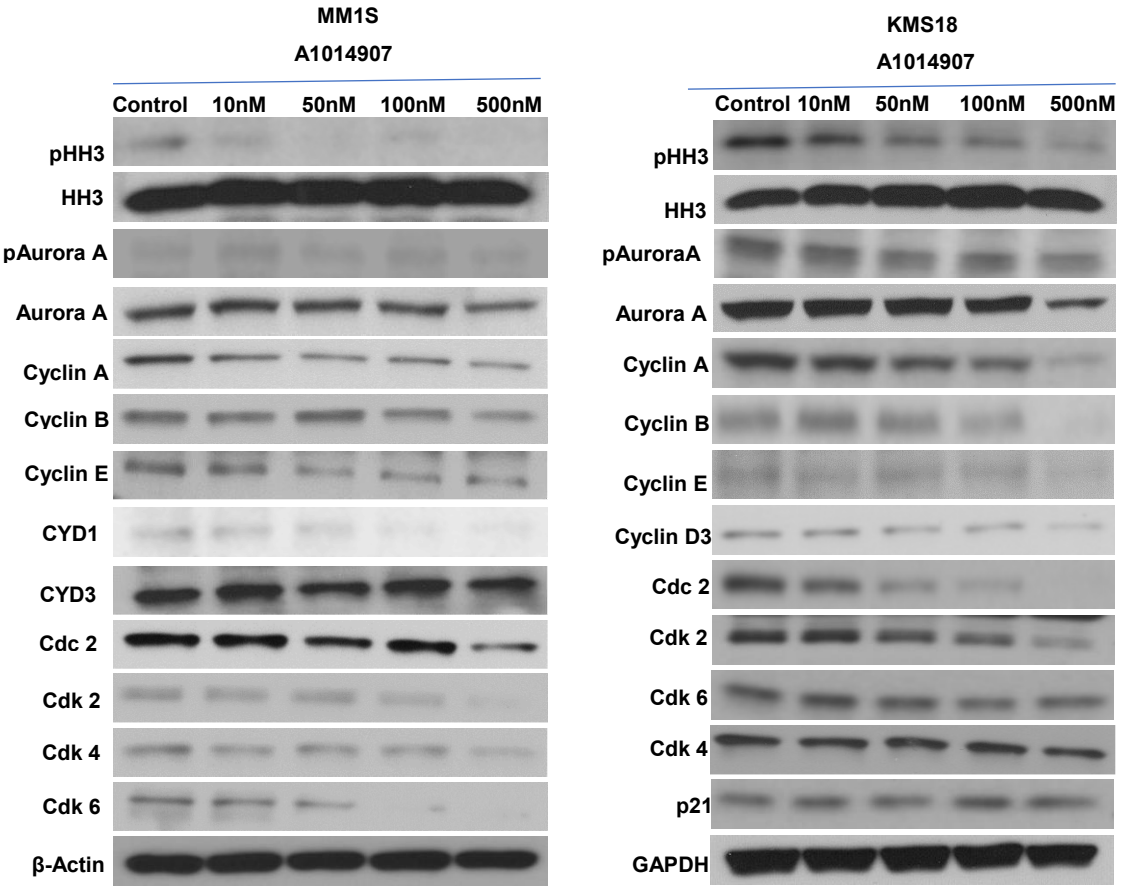


Fig 3: A1014907 causes decrease in cell cycle specific Aurora kinase A and B in MM1S and KMS18 along with reduction in cell cycle proteins



Objective IIa) - Methods and results

We reviewed the Dysproteinaemia database at Mayo Clinic, Rochester and electronic medical records, to identify patients with MM who underwent FISH testing between 2004 and August 2016 and demonstrated del (17p) at diagnosis or within 6 months of the diagnosis of MM. De novo del(17p) was defined as del(17p13.1), which includes the p53 gene region, and/or monosomy for chromosome 17. Relative loss of 17p was defined as del(17p) in presence of trisomy or tetrasomy involving chromosome 17.

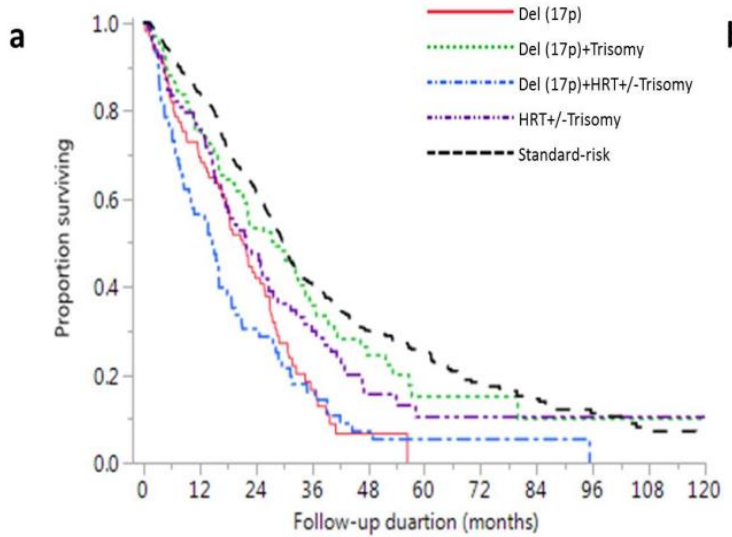
Three hundred and ten (310) patients satisfied the inclusion criteria. For each patient with del(17p), we identified two patients with MM matched for age and time period of diagnosis, which did not have del(17p) by FISH within 6 months from diagnosis and satisfied the other inclusion criteria. We subdivided the control group (n = 620) into a high-risk translocation (HRT) group [with t(4;14), t(14;16) or t(14;20)] (n = 79) and a standard- risk (SR) group (n=541) for comparing the outcomes. The data cut-off date was 31 January 2018.

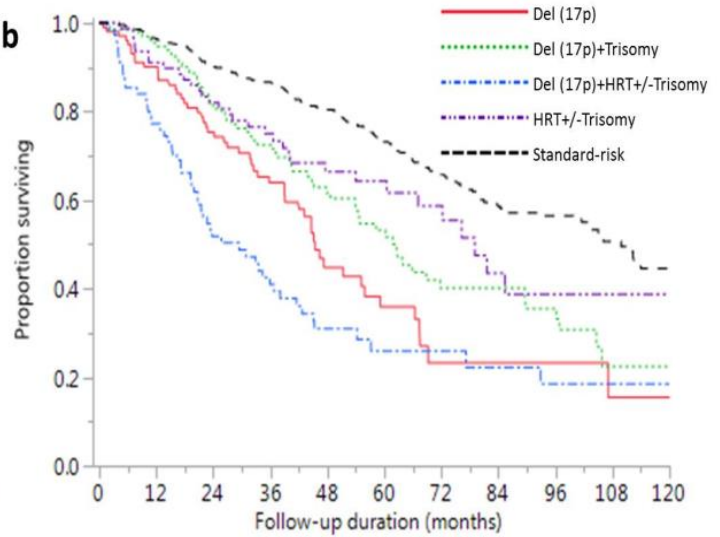
Impact on OS in presence of del17p (Fig 5)

To examine the impact of FISH abnormalities, we categorised the entire patient cohort (cases and controls) into the following groups: cases were divided into del(17p) alone (n = 135), del(17p) with hyperdiploidy (n = 100), del(17p) with high risk translocations(HRT) (irrespective of presence of hyperdiploidy) (n = 75), and controls were divided into HRT (irrespective of presence of hyperdiploidy) (n = 79) and standard risk(SR) patients (n = 541). The median PFS in these five groups were 22.4 months (95% CI, 17.8–27.0), 27.3 months (95% CI, 19.6–34.5), 14.7 months (95% CI, 9.8–17.9), 22.0 months (95% CI, 16.7–26.8), and 30.1 months (95% CI, 27.5–31.5), respectively, ($P < 0.001$)(Fig 5a). The median OS in the above five groups were 51.4 months (95% CI, 42.1–62.8), 60.3 months (95% CI, 47.8–89.6), 29.5 months (95% CI, 20.0–38.1), 79.1 months (95% CI, 60.5, not reached and 109.8 months (95% CI, 99.9-125.6), respectively ($P < 0.001$) (fig 5b).

Simultaneous hyperdiploidy improved PFS (P = 0.007) and trended toward longer OS (P = 0.272) in del(17p) patients. Coexistent HRT worsened the OS (P = 0.004).

Fig 5: a)PFS **:b)** OS of MM patients subdivided into subgroup identified by unique cytogenetically defined outcomes





Del (17p)	102	60	32	9	2	0	0	0	0	0	0
Del (17p)+Trisomy	133	87	53	31	13	5	4	2	1	1	1
Del (17p)+HRT+/-Trisomy	75	38	18	9	4	1	1	1	0	0	0
HRT+/-Trisomy	79	58	34	20	6	4	4	2	1	1	1
Standard-risk	541	425	292	162	103	68	39	24	15	8	4

Del (17p)	102	89	67	47	22	15	5	4	3	2	1
Del (17p)+Trisomy	133	126	97	74	50	34	22	20	15	4	1
Del (17p)+HRT+/-Trisomy	75	57	36	26	18	10	8	6	5	2	1
HRT+/-Trisomy	79	71	62	48	33	24	18	9	6	5	4
Standard-risk	541	514	456	397	298	214	164	117	86	54	23

Table 5: Effect of baseline characteristics on survival measures in patients with de novo del(17p) (n=310)

	Progression-free survival (PFS)			***Overall survival (OS)***		
Independent variable	p-value for univariable analysis	HR (95% CI) for multivariable analysis	p-value for multivariable analysis	p-value for univariable analysis	HR (95% CI) for multivariable analysis	p-value for multivariable analysis
ISS III vs I/II stage (93 vs. 154)	<0.001	1.92 (1.34-2.73)	**<0.001**	<0.001	2.08 (1.42-3.04)	**<0.001**
Elevated vs normal LDH (49 vs. 157)	0.001	1.71 (1.04-2.53)	**0.011**	0.003	1.83 (1.16-2.80)	**0.009**
High-risk translocation vs. no high-risk translocation (75 vs. 235)	0.001	1.44 (0.97-2.10)	0.071	<0.001	1.53 (1.01-2.27)	**0.044**

HR-Hazard ratio, HRD- Hyperdiploidy, LDH-Lactate dehydrogenase, NI- Not included in analysis, PC- plasma cell, and PI- proteasome inhibitor. The final multivariable model included 174 patients for PFS and 191 patients for OS for whom the parameters were available.

Predictors of PFS and OS (Table 5)

A univariate analysis with several disease associated patient characteristics was performed. This revealed higher ISS stage, elevated LDH and HRT with a p-value <0.1 and hence were further analysed using multivariable Cox proportional hazards model using stepwise backward elimination. The results of our final model are shown in table 5.

Objective IIb) - Methods and results

Methods and results

We reviewed the Dysproteinaemia database at Mayo Clinic, MN and identified 80 MM patients who acquired del(17p), defined as first FISH test negative for del(17p) with detection of del(17p) on a follow-up FISH test. 76 patients had relapsed or refractory MM and two had del17p reported prior stem cell transplant (SCT) after achieving some response to prior therapy line. 2 patients, other patients had incidental del17p after induction therapy prior SCT work up. These four patients were excluded to maintain homogeneity of analysed group and data on 76 patients was used in our study. FISH tests were performed for all patients between 2004 and August 2016.

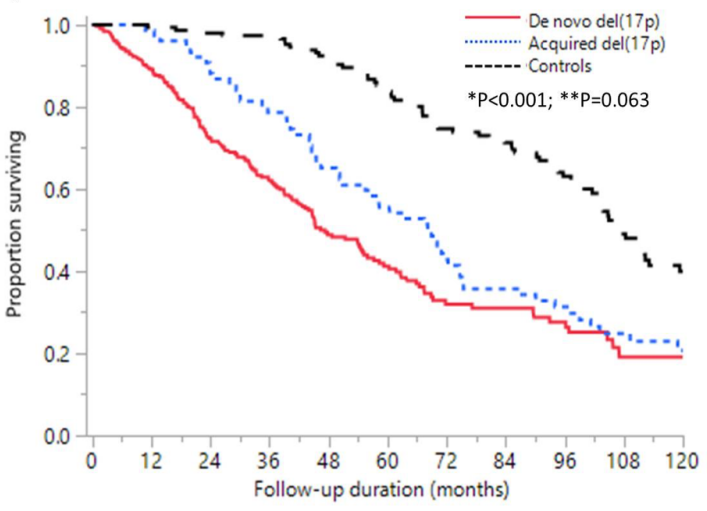
We identified 152 patients without del17p as control cohort, using two controls for each of our test cohort subject. The controls were diagnosed at overlapping period with the test cohort patient, without a notable del17p.

Lower hemoglobin (median- 10.8 g/dL vs. 11.3 g/dL; P=0.035), higher prevalence of t(4;14) among cases (15.8% vs. 6.6%; P=0.033) and increased percentage of high lactate dehydrogenase (LDH) [13.7% (7/51) vs. 4.1% (5/121); P=0.043] were noted in acquired del17p patients compared to controls.

OS of acquired del17p patients

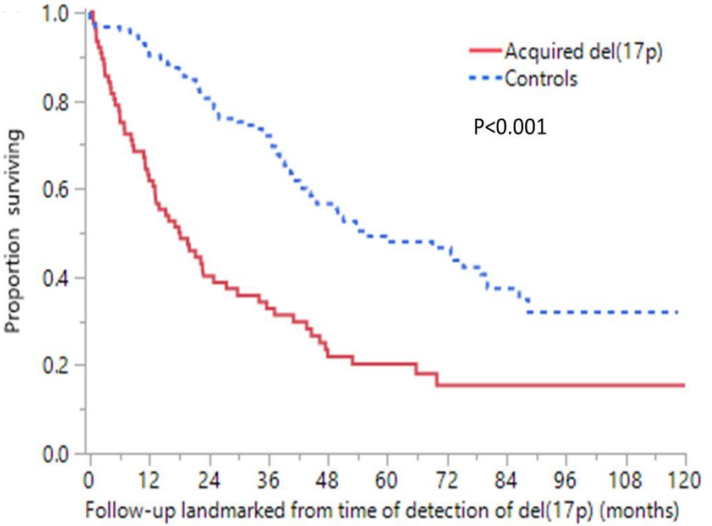
The OS of acquired cohort was 68.2 months (95% CI, 50.8-74.8), the control group 106.1 months (95% CI, 101.6-119.4) respectively (P<0.001) and 47.3 months (95% CI, 42.7-55.9) with no significant difference between the acquired vs de novo group (p=0.06) (Fig 6a). OS landmarked from time of detection of del17p in acquired del17p when compared to control patients was 18.1 months (95% CI, 11.9-25) and 56.2 months (95% CI, 44.4-79.7) respectively (P<0.001)(Fig 6b).

Fig 6a: OS from diagnosis in the 3 groups of de novo, acquired del17p and control cohort



	310	272	200	147	88	59	35	30	23	8	3
	76	75	66	58	48	40	30	25	21	13	9
	152	151	147	140	128	115	91	83	63	44	28

Fig 6b: OS landmarked from detection of del17p in acquired cohort compared to control cohort



Acquired del(17p)	76	47	28	22	14	11	5	3	3	2	1
Controls	152	137	107	89	62	38	34	17	9	1	1

Predictors of acquisition of del17p

Predictors at diagnosis of acquisition of del(17p) were identified by comparing patient characteristics in the acquired group to controls and calculating odds ratio (OR). We report a high LDH at baseline [OR- 3.69 (95% CI, 1.11-12.24)], presence of t(4;14) [OR- 2.66 (95% CI, 1.09-6.48)] and presence of an HRT [OR- 2.23 (95% CI, 1.00-4.95)] to predict for acquiring del17p in MM patients.

Discussion

Increasing evidence supports a significant impact of cytogenetic abnormalities affecting the treatment response and survival outcomes for MM patients. These abnormalities are classified as primary abnormalities which are common in MGUS stage and secondary abnormalities that increase in frequency with more active disease. The disease has 50% of patients with IgH translocations (non-hyperdiploid group), and the non-hyperdiploid abnormalities unique to the sub-clonal population of MMPC makes the disease biology complex and a clinical challenge for treatment.

We studied the role of A1014907 in MM cell lines and patient cells to identify its role in Aurora kinase inhibition. The cell cycle inhibition secondary to Aurora kinase inhibition was validated by our experiments however, we observed a dramatic difference in the sensitivity of t(4;14) containing MM cell lines when compared with the non-t(4;14) cell lines. This is unlikely to be explained merely by aurora kinase inhibition. Chang H et al. previously demonstrated 75% FGFR3 expression in t(4;14) MM patients[42].

We hypothesized a role of simultaneous inhibition of FGFR3 expression and aurora kinases in t(4;14) cell lines. Our results show that A1014907 inhibits aurora kinases and FGFR3. In cells lacking FGFR3 expression, A1014907 caused cell cycle arrest in a majority of them but failed to induce marked apoptosis. In cells expressing wild type FGFR3 or those with activating mutations in FGFR3 (Y373C or G382D), A1014907 caused potent cell cycle arrest besides pronounced apoptosis

Our pre-clinical projects highlight the natural history of MM with del17p in both, de-novo and acquired setting. Low Hb, high LDH level and PC proliferation rate, were noted in the del17p cohort. Similar observations were made by Fonseca et al. in a smaller patient cohort. Compared to a previous report of 110 patients, we observe lesser del17p with ISS III disease in our patient sample (our cohort: 36% vs 45% in the study cohort) [43]. We also show the relevance of simultaneous HRT or high LDH with del17p group, which predict for OS. This agrees with the use of these parameters when risk stratifying MM patients using revised international staging

system.

We next studied the natural history of 76 MM patients who acquired del17p after diagnosis while on treatment. The OS of this group was naturally shorter which we estimated at 18 months. These patients have been shown to do poorly in several studies with median PFS (ranging between 3.4-7.6 months) [44, 45].

An important finding of our study is a high OR for acquired del17p when certain specific parameters including, high LDH at diagnosis and presence of HRTs, especially, t(4;14). High LDH is usually a marker of aggressivity in the context of cancer biology. In solid tumours including renal cell carcinoma, melanoma and prostate carcinoma, high LDH portends poor OS and is a marker of metastatic disease[46]. High LDH levels are usually found at diagnosis and are predictive of disease relapse in hematological malignancies[47]. In MM increased LDH predicts for aggressive disease forms and reduced survival post chemotherapy[48, 49]. Hence a high LDH at diagnosis is likely a marker of advancing disease.

A previous sequential analysis of patients has shown that presence of HRTs at baseline is associated with detection of more copy number abnormalities on follow-up and this was postulated to be due to higher genomic instability in these patients.[50]. Our study is complimentary to this study as del17p is secondary cytogenetic aberration and we have shown increased odds of acquisition of del17p with pre-existing HRT's. The high risk sub-group of MM patients need more effective treatment alternatives. By identifying a simultaneous Aurora kinase and activated FGFR3 target, we have tried to address this issue for t(4;14) patients which we have also shown to improve the probability of acquiring del17p in MM patients.

Conclusions

Targeted treatment approach has significant advantages in the treatment of cancers as compared to conventional chemotherapy. Activated FGFR3 pathway is critical signalling pathway in MM cell. A1014907 is a targeted tyrosine kinase inhibitor with significant cytotoxic activity in t(4;14) cell lines. Patients with activated FGFR3 pathway are likely to benefit from A1014907.

Del17p is a poor prognosis chromosomal aberration in MM with poor OS outcomes. ISS III stage, high LDH and high-risk translocations predict for shorter OS in patients with del(17p).

del17p patients showed increased association with high baseline LDH, t(4;14) and high-risk translocations, highlighting an important association of secondary translocations in MM. This would significantly impact response to treatment as noted in our study; hence the need for newer therapeutic targets with the ability to overcome the poor prognostic impact of high-risk translocations and del17p

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Overview of publishing activities

Original publication

Lakshman A, **Painuly U**, Rajkumar V, Ketterling R, Kapoor, Griepp P,. Gertz M, Buadi F, Lacy M, Dingli D, Dispenzieri A, Fonder A, Hayman S, Hobbs M, Gonzalves W, Hwa Y, Leung N, Go R, Li Y, Kourelis T, Warsame R, Lust J, Russell S, Zeldenrust S, Kyle R, and Kumar S: Impact of acquired Del (17p) in Multiple Myeloma. Blood Advances, 2019;3(13):1930–1938.

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Painuly U, Kumar S
Efficacy of Bortezomib as First-Line Treatment for Patients with Multiple Myeloma
Clinical Medical insights: Oncology, 2013; 7:53-73. **IF 2.72**

Oral Presentation

Painuly U (May 2019) *Systemic mastocytosis in adults: 2019 update on diagnosis, risk stratification and management.*
Presented at: Haematology Journal's Club; Galway University Hospital

Painuly U (Oct 2018) *Cellular Morphology and Foundations of Genetic Hematopoietic Disorders.*
Presented at: Registrar teaching sessions: Galway University Hospital

Painuly U (Oct 2018) *Second primary malignancies in Multiple Myeloma.* Presented at: Haematology Journal's Club; Galway University Hospital

Painuly U (Sep 2018) *Clinical management of Sickle Cell Disease.* Presented at: Western Blood Club; Galway University Hospital

Painuly U (July 2018) *Safety practices and the quality in Haematology Laboratory.*
Presented at: Haematology Registrar Teaching; Galway University Hospital

Painuly U (March 2018) *Antibiotic Prophylaxis in Newly Diagnosed Multiple Myeloma.*
Presented at: Hematology Journal's Club; Galway University Hospital

Painuly U (Oct 2016) *Weekly variation in health-care quality by day and time of admission: a nationwide, registry-based, prospective cohort study of acute stroke care.*
Presented at: Grand Rounds; Sligo University Hospital

Painuly U (Jan 2016) *Diagnostic Dilemma - An unusual presentation of Pyelonephritis*.
Presented at: Grand Rounds; Sligo University Hospital

Painuly U (April 2016) *Babesiosis*.
Presented at Grand Rounds; Letterkenny

Painuly U, Cwiak M (Oct 2015) *Metastatic Cancer of Unknown Primary*
Presented at: Grand Rounds; Letterkenny

Painuly U, Rajkumar V, Ketterling R, Dispenzieri A, Lacy M, Gertz M, Buadi F, Dingli D, Hayman S, Kapoor P, McCurdy A, Leung N, Lust J, Russell S, Zeldenrust S, Lin Y, Kyle R and Kumar S. (2013) - *17p deleted Multiple Myeloma: Clinical outcomes and predictive factors for acquisition of 17p deletion.*
Presented at: American Society of Hematology conference; New Orleans, LA

Painuly U, Ramakrishnan V, Kimlinger T, Rajkumar V, Kumar S. *Promising Pre-Clinical Activity of A-1014907, a Dual Inhibitor of Aurora Kinases and VEGFR in Multiple Myeloma* Presentation at: 2013 edition the Mayo Fellows Research Day; Rochester, MN.

Poster Presentation

Lakshman A, Painuly U, Rajkumar SV, Ketterling RP, Kapoor P, Greipp P, Gertz M, Buadi F, Lacy MQ, Dingli D, Dispenzieri A, Fonder A, Hayman SR, Hobbs M, Gonsalves WI, Hwa Y, Leung N, Go RS, Lin Y, Kourelis T, Warsame R, Lust JA, Russell SJ, Zeldenrust S, Kyle RA, Kumar S *Impact of acquired del17p in patients with multiple myeloma*
Presented at: American society of hematology conference; San Diego, CA (Dec 2018)

Painuly U, Iftekar F, Rahman O, Waldron B, Walpole G, Harte P, Burke A, Hodgson A *Supportive care in Multiple Myeloma*
Presented at: Galway Research Day (June 2018)

Painuly U, Ullah K, Murray D, Harte P *Anticoagulation compliance in patients undergoing elective D/C cardioversion for Atrial fibrillation/Flutter/Paroxysmal atrial fibrillation or combination*
Presented at: International Forum on Quality and Safety in Healthcare 2018, Amsterdam (May 2018)

Paludo J, Vallumsetla N, Kumar S, Ketterling R, Gertz M, Dispenzieri A, Lacy M, Buadi F, Dingli D, Painuly U, Leung N, Kyle R, Rajkumar V and Kapoor P. *Impact of Novel Agents on Young Patients with t(11;14) Multiple Myeloma*
Presented at: The American Society of Hematology conference; San Francisco, CA (December 2014)

Gomez M, Ramakrishnan V, Prasad V, Kimlinger T, Painuly U, Bi L, Rajkumar V and Kumar S. *Overcoming Resistance to Apoptosis in Multiple Myeloma By Simultaneous Inhibition of Bcl2 and IAP Families of Anti-Apoptotic Proteins*
Presented at: The American Society of Hematology conference; San Francisco, CA (December 2014)

Lintao Bi, Ramakrishnan V, Kimlinger T, Painuly U, Rajkumar V and Kumar S. *Immunophenotyping Of Bone Marrow Stromal Cells In Multiple Myeloma And Related Plasma Cell Disorders*
Presented at: 6th Mayo Clinic Angiogenesis and Tumor Micro-environment Symposium: From Basic Science and Clinical Challenges to Patient; Rochester, MN (August 2014)

Painuly U, Rajkumar V, Ketterling R, Dispenzieri A, Lacy M, Gertz M, Buadi F, Dingli D, Hayman S, Kapoor P, McCurdy A, Leung N, Lust J, Russell S, Zeldenrust S, Lin Y, Kyle R and Kumar S. *17p Deleted Multiple Myeloma: Clinical Outcomes and Predictive Factors For Acquisition of 17p Deletion*
Presented at: The American Society of Hematology conference;
New Orleans, LA (December 2013)

Painuly U, Pandey S, Kumar S, Kyle R, Lacy M, Gertz M, Rajkumar V, Hayman S, Buadi F, Dingli D, Russell S, Lust J, McCurdy A, Dispenzieri, A and Kapoor P. *Survival Outcomes of Very Young (<40 years) Myeloma Patients.*
Presented at: The American Society of Hematology conference;
New Orleans, LA (December 2013)

Paludo J, Painuly U, Kumar S, Buadi F, Hayman S, Lacy M, Dispenzieri, A, Lust J, Dingli D, McCurdy A, Russell S, Leung N, Zeldenrust S, Rajkumar V, Gertz M, Kyle R and Kapoor P. *Myelomatous Involvement Of The Central Nervous System: Mayo Clinic Experience.*
Presented at: The American Society of Hematology conference;
New Orleans, LA (December 2013)

Kapoor P, Painuly U, Gertz M, Dispenzieri A, Lacy M, Buadi F, Dingli D, Hayman S, Russell S, Witzig T, Zeldenrust S, Leung N, Lin Y, McCurdy A, Greipp P, Kyle. R. *Outcomes of Young Patients (50 years or below) with Multiple Myeloma.*
Presented at: 14th International Myeloma Workgroup; Kyoto, Japan (April 2013)

Painuly U, Ramakrishnan V, Kimlinger T, Rajkumar V, Kumar S. (2012). *Promising Pre-Clinical Activity of A-1014907, a Dual Inhibitor of Aurora Kinases and VEGFR in Multiple Myeloma.*
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Atlanta, GA (December 2012)

Ramakrishnan V, Kimlinger T, Halling T, Haug J, Painuly U, Rajkumar V, Kumar S. *Preclinical Evaluation of AT219, a Small Molecule Inhibitor of MDM2 As an Anti-Myeloma Agent.*
Presented at: The American Society of Hematology conference;
Atlanta, GA (December 2012)

Ramakrishnan V, Kimlinger T, Painuly U, Haug J, Rajkumar V, Kumar S. (2012). *Simultaneous Inhibition of cIAP1, cIAP2 and XIAP Is Required for Inducing Apoptosis in Multiple Myeloma Cells.*
Presented at: The American Society of Hematology conference; Atlanta, GA (December 2012)