Combination Carfilzomib and the Viral Oncolytic Agent Reolysin in Patients with Relapsed Multiple Myeloma: A Pilot Study Investigating Viral Proliferation

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Introduction

- Reolysin is the infusible form of Reovirus (RV) Serotype 3 – Dearing Strain
- RV is a naturally occurring, ubiquitous, non-enveloped human reovirus associated with no or mild symptoms in humans
- Phase I clinical trials in advanced solid malignancies have consistently shown that Reolysin is safe and well-tolerated
- Our phase 1 trial investigating single agent Reolysin in patients with relapsed MM confirmed tolerability in this population, and correlative analyses showed that RV selectively entered MM cells but did not actively proliferate, findings that were associated with no objective responses
- We and others have shown that preclinically the combination of RV and Velcade or Carfilzomib (CFZ) synergistically increases MM cell killing via ER-stress mediated apoptosis
- Our group is the first to investigate Reolysin-based combination regimens in relapsed multiple myeloma patients

Methods

Inclusion criteria:
- Relapsed or refractory MM fitting or that did fit the IMWG diagnostic criteria for symptomatic disease (new or worsening end organ damage is not required for eligibility)
- Prior lenalidomide and bortezomib and disease progression within 60 days of the most recent therapy
- ECOG Performance Status ≤ 2 and age ≥ 18
- In the expansion cohort, at least 3 patients must be Carfilzomib-refractory
- Dialysis-dependent patients are eligible, but adequate marrow (ANC ≥ 1000/uL, plt count ≥ 50,ooo/uL) and liver function required

Dose-limiting toxicities:
- The Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v.4) was used to grade toxicities
- A DLT was defined as a toxicity occurring during the first cycle of therapy that was determined to be possibly, probably or definitely related to Reolysin and/or CFZ
- g4 neutropenia lasting ≥ 5 days, g3/4 thrombocytopenia associated with bleeding requiring plt transfusion, g2 or higher heart failure or LVEF < 50%, and any g3/4 non-hematologic adverse event

Correlative studies:
- Staining for reovirus RNA and protein (biomarker of viral proliferation), and apoptosis (caspase-3) will be conducted using the Leica BOND MAX immunostainer. Quantitative analysis will be performed using Ventana Vias and Caliper Biosystems Nuance.

Treatment plan:
- Patients will be treated with RV+CFZ+Dex days 1, 2, 8, 9, 15 and 16 of a 28-day cycle, unless MR or better is evident after cycles 4 and 11, then weekly or biweekly dosing, respectively, can be considered to increase tolerability

Dose level	Dexamethasone (IVP)	Carfilzomib (IVPB)	Reolysin (IVPB)
-1	20 mg/day	20 mg/m^2/day	3 x 10^9 $TCID_{50}$/day
1 (starting)	20 mg/day	C1 Days 1 & 2 – 20 mg/m^2/day C1 Day 8 & onward – 27 mg/m^2/day	3 x 10^{10} $TCID_{50}$/day

Patients

Patients have been enrolled to date, 8 are evaluable for toxicity and response

ID	Age	Sex	Race	Dialysis?	Myeloma	ISS Stage	Karyotype	Cytogenetics
1	64	F	C	N	IgG	2	NL	NL
2	70	F	C	N	KLC	2	Complex	(+)1q21
3	59	M	C	N	LLC	2	Complex	Del13q, +1q21, t(11;14), +1p36, +1q23
4	66	F	C	N	IgG	2	NL	del13q
5	68	M	C	N	IgG	1	NA	NA
6	63	M	C	N	LLC	2	NL	del13q, t(14;16), del17p
7	43	M	C	Y	LLC	3	NA	t(4;14), del13q
8	55	F	C	N	IgG	1	del13q, 1p-	t(4;14)
9	53	M	C	N	LLC	3	NL	(+)1q21, t(11;14), del13q

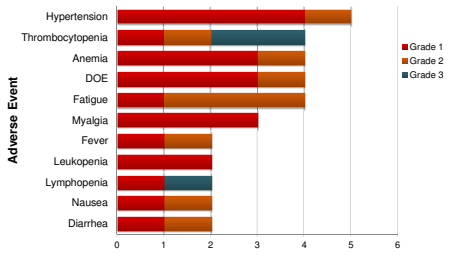
Median age is 63 (range 43-70), all are Caucasian, one was dialysis-dependent.
At diagnosis, median ISS stage was 2 (range 1-3), 5 patients had intermediate or high-risk disease.

ID	CFZ-sensitive	CFZ-exposed	Rev-exposed	Vel-exposed	Vel-refractory	# Prior lines Rx	# Prior Rx	Prior Rx
1	Y	N	Y	Y	N	1	3	Velcade/Rituximab/Dex/Cy, autoHSCT, Len
2	Y	N	Y	Y	Y	2	4	Rd, autoHSCT, R, Vd
3	Y	Y	Y	Y	Y	6	8	Vd, autoHSCT, VRd, Vd, AR-42, Cd, CCd, Pd
4	Y	N	Y	Y	N	1	3	VRd, autoHSCT, Rcy
5	Y	N	Y	Y	N	3	4	Vd, VRd, CyD, VD-PACE
6	Y	N	Y	Y	Y	2	4	Rd, VRd, autoHSCT, VRd
7	Y	N	Y	Y	Y	2	5	Vd, VRd, Cy, autoHSCT, Rp
8	Y	N	Y	Y	N	1	2	RVDD, autoHSCT
9	Y	N	Y	Y	N	2	3	Vd, autoHSCT, Isatuximab-Rd

All patients are CFZ sensitive and have been treated with Rev, 1 is CFZ-exposed, 4 are Vel-refractory.
Median # prior lines of therapy is 2 (range 1-6) and median # of treatments is 4 (2-8).

Toxicities

Cycle 1 AEs possibly, likely, or definitely attributable to Reolysin and/or Carfilzomib experienced by the 8 evaluable patients

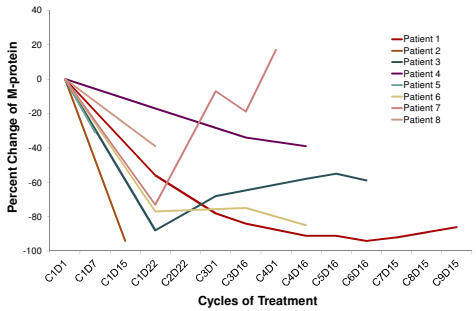


Total Number of Worst per Patient Events by AE Category Subdivided by Toxicity Grade

- Patients have evidence of HTN, cytopenias, and flu-like symptoms
- 2 patients experienced DLTs including myocarditis (g4), LV dysfunction (g3), and respiratory failure (g4) possibly attributable to RV+CFZ, and LGIB not attributable to combination treatment
- No other g3 events occurred, other g2 events include chills and bone pain in one patient, and other g1 events include HA, weakness, and parasthesia (all n =1)

Response

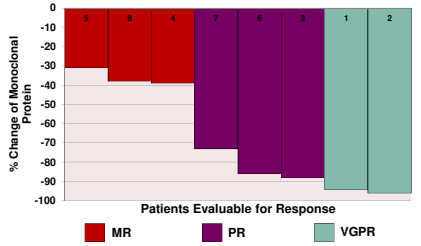
Spider plot showing trend of dominant monoclonal protein over the course of treatment



Five of 8 patients remain on study, 2 patients experienced a DLT after 2 doses, and 1 dialysis-dependent patient treated at dose level -1 progressed after 3 cycles

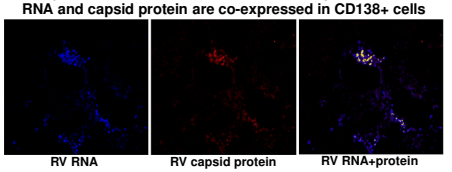
ID	Total Cycles to date	Best Response	Currently treated	If removed, how many total doses?	Reason for removal
1	9	VGPR	Y	NA	NA
2	1	VGPR	N	2	GIB
3	6	PR	Y	NA	NA
4	4	MR	Y	NA	NA
5	1	MR	N	2	Acute cardiomyopathy
6	4	PR	Y	NA	NA
7	3	PR	N	3 cycles	Progression
8	1	MR	Y	NA	NA

Waterfall plot illustrating that all evaluable patients have MR or better

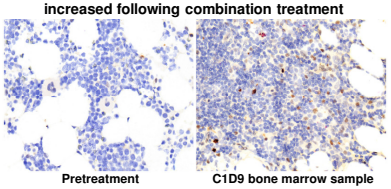


Correlatives

In situ hybridization of C1D9 bone marrow samples show that RV RNA and capsid protein are co-expressed in CD138+ cells



Caspase-3, a marker of apoptosis, is significantly (p=0.005) increased following combination treatment



Conclusions

- Our group is the first to investigate a Reolysin-based combination regimen in patients with relapsed MM
- Treatment has been relatively well tolerated, most patients experience flu-like symptoms over the 1st week of treatment, and cytopenias, especially thrombocytopenia are evident
- 2 DLTs were evident in the 1st cohort of CFZ-sensitive patients, one for myocarditis and subsequent LV dysfunction and respiratory failure possibly attributable to RV+CFZ, and the other for LGIB, not attributable to combination treatment
- All patients have achieved MR or better, and only one patient has progressed (following 3 cycles of treatment)
- Preliminary correlative studies indicate a higher degree of viral RNA, capsid protein, and apoptosis following RV+CFZ than was evident in our single agent RV trial
- Exploratory analyses from our single agent RV and combination RV+CFZ trials show that compared to RV alone, treatment with RV+CFZ is associated with upregulation of PD-L1 on CD138+ plasma cells and increased infiltration of CD8+ T-cells. Further, following RV+CFZ, both PDL1 and caspase-3 are significantly increased (p=0.005). These interesting findings necessitate continued investigation, and suggest that the addition of a PD1 or PDL1 inhibitor may further optimize the RV+CFZ regimen.

Acknowledgements

This clinical trial was sponsored by CTEP under U01-CA76576 (PI Grever). The content is solely the responsibility of the authors and does not represent the official views of the National Cancer Institute or the National Institutes of Health.