Human Safety Testing of Subcutaneously-administered Ex-RAD® (ON 01210.Na), a Small Molecule Radioprotection Agent

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Abstract

Background: Threat of radiation exposure during natural or manmade disasters underpins the search for medical countermeasures to acute radiation injury. Ex-RAD® (recilisib sodium, ON 01210.Na) is being developed by Onconova Therapeutics as a novel radiation protection agent. When dosed either 24 h pre- or post-radiation, *Ex-RAD* provided enhanced survival and rate of hematopoietic recovery in mice. FDA's "Animal Rule" requires safety evaluation in healthy subjects, with efficacy testing only in animals. Here, we report results of two Phase I clinical studies of subcutaneously (SC)-administered *Ex-RAD* in healthy adults.

Methods: First-in-man study was a randomized, placebo-controlled single ascending dose trial of 50, 100, 200 or 300 mg of *Ex-RAD* (N=32 subjects). Inflammatory serum cytokines (IL-2, IL-6, IL-10, TNF-α, IFN-γ, MCP-1) were monitored.

The second randomized, placebo-controlled trial (N=20) evaluated a fractionated 2-dose regimen (200 & 400 mg total doses) and compared absorption kinetics from SC injection sites (abdomen, thigh, buttock).

Results: In both studies, *Ex-RAD* was well-tolerated, without clinically significant drug-related systemic toxicity. Main adverse events were mild, self-limited injection site reactions, generally subsiding in a few hours. No clinically-significant trends were noted in plasma cytokines between the *Ex-RAD* and placebo-treated groups.

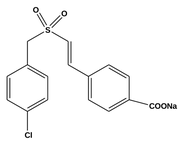
In Study 1, *Ex-RAD* was readily absorbed after a single injection, with median T_{max} values from 1.5 - 2.0 h and apparent mean elimination $t_{1/2}$= 1.78 - 3.81 h (50-200mg) and 13.3 h (300mg, biphasic elimination). Dose exposure increased proportionately across the full 50-300mg range.

In Study 2, split SC doses were administered 4 h apart. *Ex-RAD* was readily absorbed and eliminated from plasma for both 200 and 400 mg dosings (median T_{max} 1.5 h for both dose levels and intervals); C_{max} and AUC appeared to increase less than dose-proportionally between 200 and 400 mg dose levels for each interval examined, with corresponding increases in CL/F and Vz/F. Rate of absorption was similar whether injections were in abdomen, thigh or buttock sites.

Conclusions: *Ex-RAD* administered SC to 40 healthy adults showed a good safety profile and rapid absorption, suggesting feasibility for emergency use for war fighters, first-responders, and potentially wider at-risk populations.

Background

Ex-RAD® (Recilisib sodium, ON 01210.Na)



- Novel small molecule drug being developed by Onconova Therapeutics, Inc. as a medical radiation countermeasure for prophylactic use;
- Increased survival, more rapid hematopoietic recovery and protection of GI tissues have been observed in mice following prophylactic dosing;[1,2]
- Oral or SC administration of *Ex-RAD* is effective in mice.[3]

Methodology

- Studies were conducted at Covance Clinical Research Unit (Evansville, IN) following GCP regulations, with informed consent and IRB oversight;
- Randomized, double-blind, placebo-controlled trial design;
- Males or females, 18-50 years of age, with body mass index of 19-30 kg/m^2, inclusive; non-smoker, in good health, based upon results & medical history;
- Safety evaluation included adverse event (AE) & injection site assessments, 12-lead ECGs, vital signs, physical examinations and laboratory assessments;
- A validated LC-MS/MS assay was used for plasma drug determinations; non-compartmental analysis to determine pharmacokinetic (PK) parameters.

First-in-Man Clinical Trial (Study #1):

- Single ascending doses in 32 healthy adult volunteers, as 4 Cohorts (N=8);
- Subjects in each Cohort randomized to receive *Ex-RAD* (N=6) or placebo (N=2);
- Subjects received 50 mg, 100 mg, 200 mg or 300 mg of *Ex-RAD* as SC injections (or matching placebo injections), distributed over different locations: buttocks, thighs and lower abdomen;
- Cohort 4 subjects (receiving 300-mg or placebo) evaluated for selected plasma cytokines (IL-6, IL-10, TNF-α, IFN-γ, IL-2 and MCP-1) using a multiplex immunoassay; drug responses were compared to placebo & historical controls.

Fractionated SC Dosing Clinical Trial (Study #2):

- Fractionated doses, 4 hr apart, in 20 healthy adults; 2 Cohorts (N=10);
- Subjects in each Cohort (N=10) were randomized to two dosing sequences of *Ex-RAD* (N=4) or placebo (N=1);
- Subjects received 200 mg or 400 mg total doses of *Ex-RAD* (or matching placebo) as SC injections, in 2 sequences in buttocks, thighs and lower abdomen.

First-in-Man Clinical Trial

Study Objectives

Primary objectives:

- Determine local tolerability of single ascending doses of subcutaneously (SC)-administered *Ex-RAD*;
- Determine systemic safety of single ascending doses of SC-administered *Ex-RAD*;
- Determine maximum tolerated dose of SC-administered *Ex-RAD*;
- Determine PK behavior of single ascending doses of SC-administered *Ex-RAD*.

Secondary objective:

- Evaluate the effect of *Ex-RAD* on selected serum cytokines.

Study #1 Results

Pharmacokinetics:

- *Ex-RAD* was readily absorbed (T_{max} about 1.5 – 2 hr), with rapid plasma clearance;
- Drug exposure increased with increasing dose; (Figure 1 & Table)

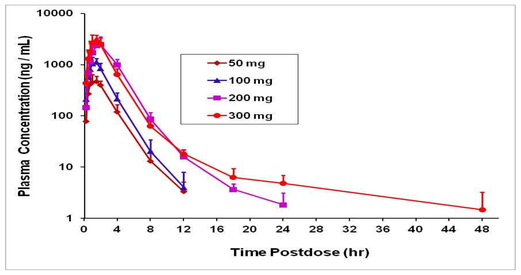


Figure 1: Arithmetic mean of *Ex-RAD* (ON 01210) plasma concentrations *vs.* time after SC injections at different doses (Semi-logarithmic scale).

Mean (SD) PK Parameters for Clinical Study #1

Parameter	Units	*Ex-RAD* Dose Level (mg) 50	100	200	300
C_{max}	(ng/mL)	**497** (166)	**1157** (252)	**2603** (1026)	**2983** (1028)
T_{max}[a]	(hr)	**1.50** (0.75, 2.00)	**1.50** (1.00, 1.50)	**2.00** (1.5, 2.00)	**1.50** (1.00, 1.50)
$AUC_{0-\infty}$	(ng*hr/mL)	**1532** (318)	**3161** (677)	**8726** (2792)	**8876** (2982)
λ_z	(1/hr)	**0.354** (0.155)	**0.465** (0.204)	**0.213** (0.077)	**0.088** (0.081)
$t_{1/2}$	(hr)	**2.47** (1.51)	**1.78** (0.81)	**3.81** (2.02)	**13.3** (8.33)
CL/F	(L/hr)	**34.0** (7.76)	**33.1** (8.11)	**25.0** (8.32)	**36.5** (9.97)
V_z/F	(L)	**128** (107)	**83.0** (36.1)	**133** (58.8)	**642** (351)
DN-$AUC_{0-\infty}$	(ng*hr/mL)/(mg)	**30.6** (6.36)	**31.6** (6.77)	**43.6** (14.0)	**29.6** (9.94)
DN-C_{max}	(ng/mL)/(mg))	**9.95** (3.32)	**11.6** (2.52)	**13.0** (5.13)	**9.94** (3.43)

[a] Median (Min, Max) presented for T_{max}; DN = Dose-Normalized parameter

Safety:

- SC-administered *Ex-RAD* was well-tolerated with no serious adverse events (AE) reported;
- Maximum tolerated dose for SC-administered *Ex-RAD* was not determined;
- Mild injection site reaction was the major AE reported for both *Ex-RAD* & placebo;
- Local irritation at the site of injection generally resolved within a few hours;
- Levels of plasma cytokines did not show significant drug- or time-dependence .(See Table)

Plasma Cytokine Levels for Cohort 4 Subjects (pg/mL)*

Subject ID	Time	IL-6	IL-10	TNF-α	IFN-γ	IL-2	MCP-1
25	Predose	4.3	2.4	BQL[a]	BQL[b]	BQL[c]	210.3
	4hr	11.9	4.3	BQL	BQL	BQL	194.8
	12hr	25.1	3.7	BQL	BQL	BQL	229.5
	24hr	23.8	4.0	BQL	2.1	BQL	256.5
26	Predose	23.0	11.9	BQL	20.3	BQL	515.0
	4hr	26.9	11.3	BQL	24.7	BQL	541.9
	12hr	34.9	13.5	BQL	15.6	BQL	572.8
	24hr	34.0	9.8	BQL	17.6	BQL	422.5
27 (Placebo)	Predose	9.1	4.8	BQL	BQL	BQL	277.3
	4hr	28.0	4.6	BQL	BQL	BQL	483.4
	12hr	54.5	6.0	BQL	BQL	BQL	530.0
	24hr	82.3	16.7	BQL	BQL	BQL	386.4
28 (Placebo)	Predose	6.8	3.3	BQL	BQL	BQL	344.3
	4hr	16.1	3.2	BQL	BQL	BQL	286.4
	12hr	141.8	2.8	BQL	BQL	BQL	423.1
	24hr	13.7	5.0	BQL	BQL	BQL	413.7
29	Predose	5.2	3.3	BQL	8.3	BQL	379.6
	4hr	25.5	3.3	BQL	13.0	BQL	372.7
	12hr	181.4	3.3	BQL	3.0	BQL	823.8
	24hr	10.6	5.5	BQL	22.0	BQL	286.5
30	Predose	4.2	5.8	BQL	59.6	2.2	280.5
	4hr	6.5	8.1	BQL	75.4	BQL	257.9
	12hr	6.4	4.7	BQL	62.8	2.4	227.3
	24hr	9.0	7.5	BQL	61.1	2.4	228.6
31	Predose	6.1	7.7	BQL	19.7	BQL	294.1
	4hr	9.5	6.3	BQL	33.2	BQL	317.6
	12hr	5.4	4.7	BQL	25.8	BQL	280.7
	24hr	5.4	5.5	BQL	28.9	BQL	265.9
32	Predose	14.2	11.8	BQL	41.6	BQL	305.9
	4hr	10.0	9.8	BQL	49.7	BQL	249.0
	12hr	9.8	4.0	BQL	20.4	BQL	228.8
	24hr	23.1	11.5	BQL	51.1	BQL	207.5

[a] Below Quantitation Level (BQL) = <11.5 pg/mL for TNF-α [b] BQL = <2.0 pg/mL for IFN-γ
[c] BQL = <2.0 pg/mL for IL-2 Note: Highlighted values above historical normal range.
Historical ranges: IL-6 (BQL - 138.0 pg/mL); MCP-1 (249.7 - 616.1 pg/mL)
*SearchLight Multiplex immunoassays; Aushon BioSystems (Billerica, MA)

Clinical Study #2

Study Objectives

Primary objectives:

- Determine the local tolerability of a 2-dose regimen of SC-administered *Ex-RAD*;
- Determine systemic safety of a 2-dose regimen of SC-administered *Ex-RAD*;
- Determine PK behavior of SC-administered *Ex-RAD u*nder the conditions of the trial.

Secondary objective:

- Estimate effect of injection site on relative bioavailability of *Ex-RAD*.

Study #2 Results

Pharmacokinetics:

- *Ex-RAD* was readily absorbed, independent of the site of SC administration;
- Drug exposure increased in a less-than-dose-proportional manner from 200 to 400 mg doses;
- Sites of injection did not affect bioavailability, drug exposure or kinetics; (Figure 2 & Tables)

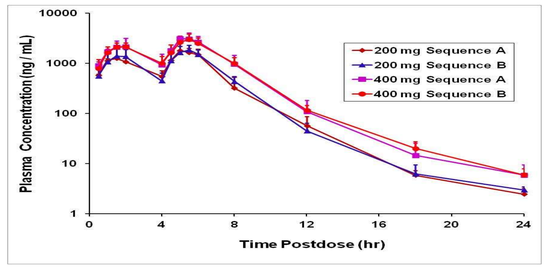


Figure 2: Arithmetic mean of *Ex-RAD* (ON 01210) plasma concentrations *vs.* time after 2 SC injections at different doses and dose sequences (Semi-logarithmic scale).

Dosing	**Cohort 1** (200 mg total)	**Cohort 2** (400 mg total)
Sequence A (4 active, 1 placebo)	Dose 1: 50 mg each buttock Dose 2: 50 mg each side of abdominal wall	Dose 1: 100 mg each buttock Dose 2: 50 mg each side of abdominal wall and each thigh
Sequence B (4 active, 1 placebo)	Dose 1: 50 mg each side of abdominal wall Dose 2: 50 mg each buttock	Dose 1: 50 mg each side of abdominal wall and each thigh Dose 2: 100 mg each buttock

Mean (SD) Plasma PK Parameters by Dose Sequence & Dosing Interval

	Ex-RAD Dose Level (total mg) 200 mg (Cohort 1)		400 mg (Cohort 2)	
Parameter (Units)	Sequence A	Sequence B	Sequence A	Sequence B
First Dosing Interval				
C_{max1} (ng/mL)	**1258** (105)	**1535** (579)	**2248** (932)	**2243** (506)
T_{max1} [a] (hr)	**1.50** (1.50, 1.50)	**1.75** (1.50, 2.00)	**1.50** (1.50, 2.00)	**1.75** (1.50, 2.00)
AUC_{0-t} (ng*hr/mL)	**3372** (458)	**3639** (1372)	**5943** (2187)	**5910** (1162)
Second Dosing Interval				
C_{max2} (ng/mL)	**1995** (857)	**1930** (489)	**3348** (735)	**3118** (853)
T_{max2} [a,b] (hr)	**5.25** (5.00, 5.50)	**5.50** (5.00, 5.50)	**5.25** (5.00, 5.50)	**5.50** (5.00, 5.50)
$t_{1/2}$ (hr)	**2.74** (0.363)	**2.78** (0.243)	**2.94** (0.320)	**2.80** (0.339)
λ_z (1/hr)	**0.256** (0.0301)	**0.251** (0.0232)	**0.238** (0.0238)	**0.250** (0.0303)
Total Dosing Interval				
C_{max} (ng/mL)	**1995** (857)	**1930** (489)	**3348** (735)	**3118** (853)
CL/F (L/hr)	**22.9** (4.52)	**21.8** (5.79)	**25.0** (7.21)	**24.8** (5.40)
V_z/F (L)	**91.3** (26.6)	**88.7** (30.2)	**108** (42.3)	**101** (26.6)
$AUC_{0-\infty}$ (ng*hr/mL)	**9048** (2137)	**9603** (2266)	**17201** (5585)	**16775** (3838)
DN AUC_{0-t} [(ng*hr/mL)/mg]	**45.2** (10.7)	**48.0** (11.3)	**42.9** (14.0)	**41.9** (9.60)
DN $AUC_{0-\infty}$ [(ng*hr/mL)/mg]	**45.2** (10.7)	**48.0** (11.3)	**43.0** (14.0)	**41.9** (9.59)
DN $C_{max\ (total)}$ [(ng/mL)/mg]	**9.98** (4.28)	**9.65** (2.45)	**8.37** (1.84)	**7.79** (2.13)

[a] Median (min, max) shown for T_{max}; [b] T_{max2} is presented as the time from the first dose.

Safety:

- Two-dose SC regimen of *Ex-RAD* was generally well tolerated, with no serious AE reported;
- Injection site reaction was the most commonly reported AE for both *Ex-RAD* & placebo;
- All AEs were mild or moderate and resolved prior to the end of the study;
- No clinically-significant systemic toxicity, laboratory or safety-related findings.

Conclusions

- SC dosing of *Ex-RAD* was generally well tolerated and did not induce inflammatory plasma cytokines, with no reports of serious AEs;
- Mild injection site reaction was most commonly reported AE for both *Ex-RAD* & placebo;
- No clinically-significant systemic toxicity, laboratory, or safety-related findings
- *Ex-RAD* was readily absorbed (T_{max} about 1.5 – 2 hr), with rapid plasma clearance;
- Rapid absorption suggests feasibility of SC use of *Ex-RAD* to protect war fighters, first-responders and potentially wider populations at risk for harmful radiation exposure.

References

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For more information about Onconova Therapeutics and Ex-RAD®: **http://www.onconova.com**