A Mechanism of Action Study of Intra-tumoural or Intravenous Dosing of Enadenotucirev, an Oncolytic Adenovirus, in Patients with Colon, Lung, Bladder and Renal Carcinoma Undergoing Resection of Primary Tumour

Rocio Garcia-Carbonero[1], Valentina Boni[2], Ignacio Duran[1], Marta Gil-Martin[3], Marta Espinosa[1], Ramon Salazar[3], Antonio Cubillo[2], Monica Jurado[4], Brian Champion[5], Simon Alvis[5], Kerry Fisher[5], John Beadle[5], Gillian Over[5], Hilary McElwaine-Johnn[5], Christopher Ellis[5], Christine Wilkinson-Blanc[5], Emiliano Calvo[2]

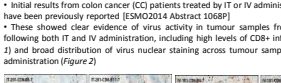
1. Hospital Universitario Virgen del Rocio, Seville, Spain, 2. START Madrid, Centro Integral Oncológico Clara Campal, Hospital Madrid Norte Sanchinarro, Madrid, Spain, 3. Early Clinical Research Unit, Institut Català D´Oncologia, L´Hospitalet-Barcelona, Barcelona, Spain, 4. Pivotal, Gobelas, 19. La Florida 28023 Madrid, Spain, 5. PsiOxus Therapeutics Limited, Milton Park, Abingdon, United Kingdom

Abstract 1087P

Clinical Trial Information: EUDRACT 2013-000562-11

1. BACKGROUND

- Enadenotucirev (EnAd) is a tumour selective chimeric Ad11/Ad3 group B adenovirus that has demonstrated preclinical activity in a metastatic model of colorectal cancer and in human tumour biopsies ex-vivo[1]
- To date, clinical success with oncolytic viruses has tended to be associated with intra-tumoural (IT) administration. Demonstrating delivery to tumours in patients by intravenous (IV) dosing is key for directing the development of EnAd
- Serological studies suggest that the prevalence of neutralising antibodies against group B adenoviruses is low[2] which may permit systemic delivery of EnAd. Pre-clinical work has shown that EnAd retains oncolytic activity in the presence of fresh whole human blood[3]
- Initial results from colon cancer (CC) patients treated by IT or IV administration of EnAd have been previously reported [ESMO2014 Abstract 1068P]
- These showed clear evidence of virus activity in tumour samples from CC subjects following both IT and IV administration, including high levels of CD8+ infiltration (*Figure 1*) and broad distribution of virus nuclear staining across tumour samples following IV administration (*Figure 2*)



Note: Figures are labelled with Patient Number-Stain-Block Number-Section Number

Figure 1: Examples of CD8+ Staining for Colon Cancer Patients following A) IT Injection; B) IV Infusion of EnAd

Figure 2: Examples of Distribution of EnAd Nuclear Staining across all Sections from all Tumour Sample Blocks for Colon Cancer Patients following A) IT Injection; B) IV Infusion

Following completion of these first two cohorts of patients with CC, the study was extended to enable assessment of IV delivery of EnAd to other tumour types. Here, we report further results of the study, including an expansion to include lung, bladder and renal cancer patients.

References

1.Kuhn et al, PLoS ONE 2008; 3 (6):e2409;
2.Holterman et al, J Virol. 2004 Dec; 78(23):13207-15;
3.Y Di et al. Gene Therapy. 2014; 21, 440-443

2. STUDY DESIGN & METHODOLOGY

This was a Phase I multicentre, non randomised, open label, mechanism of action clinical study to investigate administration of EnAd in a pre-operative setting. It was designed to assess the pattern of viral delivery, viral expression and inflammatory infiltrates within tumour tissue, normal tissues and draining lymph nodes following administration of EnAd to patients with histologically confirmed cancer, scheduled for surgical removal of primary tumour. The primary objective was to describe EnAd delivery to tumour cells. Immunohistochemistry (IHC) staining of formalin fixed (FFPE) sections for EnAd hexon protein (produced late during viral replication) was used to visualise virus activity. A quantitative polymerase chain reaction (qPCR) specific to EnAd was used to detect virus genome. Further IHC studies were conducted with a panel of immune markers and gene expression was analysed using RNA extracted from FFPE sections (Nanostring). Nanostring analysis is being used to assess immune gene expression levels in RNA samples extracted from FFPE sections. RNA samples prepared from sections of 5 different untreated CC tumour samples were run as controls.

Methodology

Five cohorts of patients with histologically confirmed cancer were treated; two cohorts of colon cancer patients and three cohorts of patients with other tumour types: non-small cell lung cancer [NSCLC], urothelial cell carcinoma [UCC] or renal cell carcinoma [RCC], Cohorts A, B, C, D and E, respectively.

All patients were screened within 5 days before EnAd administration. Eligible patients then received the study treatment as summarised in the table below, underwent surgery and were thereafter followed over 22 days for CC patients or 33 days for patients with other tumour types. The final follow up visit took place 56 days after last dose i.e. Day 56 in IT and Day 61 in IV groups, or 28 days after surgery if this was later.

Enadenotucirev Administration

Cohort A	$1x10^{11}$ vp/ml with a range of 0.6 to 3.0 $x10^{11}$ total vp IT on Day 1 (a variable volume of EnAd was injected based on the tumour surface area)
Cohorts B-E	$1x10^{12}$ vp IV over 5 min on Days 1, 3 and 5 (N=12)*

Abbreviations: vp = viral particles; IT=intra-tumoural; IV=intravenous; *One subject received only 2 doses (R301)

Patient Characteristics

A total of 17 patients were recruited to the study: 10 CC (5 IT/5 IV); 2 NSCLC; 2UCC and 3 RCC (all IV). Surgery was between Days 8 and 51.

3. SAFETY AND TOLERABILITY

Administration of EnAd was generally well tolerated. All TEAEs were of maximum CTCAE Grade 1 or 2 severity in three (60.0%) patients following IT injection and nine (75.5%) patients following IV infusion. There were no treatment related TEAEs of CTCAE Grade 3 or 4 severity. Four patients had non-treatment related SAEs in the study, the incidence was comparable following IT injection and IV infusion. One patient with RCC had a non-treatment related TEAE leading to study treatment discontinuation.

Commonly reported treatment related TEAEs following IV infusion (Cohort B-E) were asthenia (33%); chills (25%); neutropenia (25%); pyrexia (25%). No TEAEs were reported as treatment related following IT injection.

4. RESULTS

Histopathology and Immunohistochemistry

Extensive IHC assessment was performed in the 10 CC patients treated in Cohorts A & B (5 IT/5 IV). Clear evidence of virus activity (punctate brown staining of nuclear hexon protein) was found in tumour samples from all 10 patients.

Table 1 Summary of Percentage EnAd Nuclear Staining of Sections of Resected Tumour Tissue of Colon Cancer Patients (FAS; Cohorts A and B)

Cohort	Patient	Total Number of Sections	% Nuclear Staining					
			No Staining	>0-≤20	>20-≤40	>40-≤60	>60-≤80	>80-≤100	No Tumour
A (IT)	IT0201	42		31	8	3			
	IT0203	14				2	11	1	
	IT0204	42	1				4	30	7
	IT0301	21	7	14					
	IT0302	28	14	11	2	1			
	TOTAL	**147**	**22**	**56**	**10**	**6**	**15**	**31**	**7**
B (IV)	IV0101	49		38	8	3			
	IV0201	21			3		11	7	
	IV0301	70		25	11	13	6	8	7
	IV0302	63		21	9	9	4	6	14
	IV0303	21		11	7	3			
	TOTAL	**224**	**0**	**95**	**38**	**28**	**21**	**21**	**21**

Patient IV0201 did not have surgery until 51 days after the first IV infusion of EnAd

Nine of the 10 CC patients had surgery performed between 8 and 17 days after the first dose of EnAd. The remaining patient (IV0201) had surgery delayed and virus was still detectable 51 days after the first dose of EnAd, see *Figure 3*.

There was minimal or no staining of stromal cells/normal cells in the tumour samples, and no or generally weaker staining in associated normal tissue, see *Figure 4* for an example. Where staining did occur in normal cells, there was no sign of any pathology and the cells looked healthy.

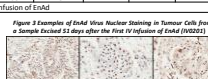


Figure 3 Examples of EnAd Virus Nuclear Staining in Tumour Cells from a Sample Excised 51 days after the First IV Infusion of EnAd (IV0201)

Figure 4 Selective Staining of Tumour Tissue compared to Normal Tissue (Patient IV0302)

Nanostring Analysis

Table 2 Preliminary Analysis of Nanostring Data Comparing Samples from Cohorts A and B to Untreated Colorectal Carcinoma Samples.

Cohort	Patient	Immune Gene Expression					
		No. of Genes ≥2xCC mean		No. of Genes ≤0.5xCC mean		Total (%) of Genes different to CC mean	
A	IT0201	22	(2.9%)	26	(3.4%)	48	(6.3%)
	IT0203	12	(1.6%)	118	(15.3%)	130	(16.9%)
	IT0204	39	(5.1%)	11	(1.4%)	50	(6.5%)
	IT0301	13	(1.7%)	54	(7.0%)	67	(8.7%)
	IT0302	19	(2.5%)	36	(4.7%)	55	(7.2%)
B*	IV0101	54	(7.0%)	46	(6.0%)	100	(13.0%)
	IV0201	5	(0.6%)	77	(10.0%)	82	(10.6%)
	IV0301	18	(2.3%)	19	(2.5%)	37	(4.8%)
	IV0302	7	(0.9%)	68	(8.8%)	75	(9.7%)

Note: Patient IV0303 did not consent to this additional exploratory analysis

Analysis of Nanostring data used the nCounter Pancancer immune gene profiling probe set of 770 genes. For each gene in the panel, the expression level for each patient sample was divided by the mean of the 5 control CC samples for that gene. The number of genes whose expression levels were either ≥ 2 or ≤ 0.5 for each of the samples for a patient were counted as genes that may be potentially regulated in response to treatment with EnAd. Data are expressed as total number and as % of the full 770 gene set, see *Table 2*.

Viral Genome Detection

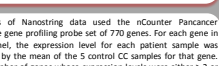


Figure 5 EnAd Genome Copies in Tumour Samples by qPCR

Exploratory qPCR analysis of EnAd genomic DNA in tumour samples from both CC patients and patients with other tumour types provided supporting evidence of IV delivery of EnAd. EnAd DNA could only be detected in two out of five tumours following IT injection. This is likely due to the more focal nature of delivery by IT injection leading to some tumour tissue pieces being taken from uninjected areas, see *Figure 5*.

5. DISCUSSION AND CONCLUSIONS

- IHC analysis showed selective delivery of EnAd to CC tumour cells
- qPCR analysis confirmed presence of EnAd in CC, UCC, NSCLC and RCC following IV infusion
- High levels of CD8+ cells infiltrating tumour cell nests were observed in 8/10 CC patients, consistent with an immune stimulation hypothesis for EnAd
- Preliminary Nanostring analysis highlighted potential treatment-responsive genes for further evaluation
- Administration of EnAd was generally well tolerated with the most commonly reported adverse events following IV infusion consistent with 'flu like symptoms as previously described

This study provides valuable information to support future clinical studies of IV dosing of EnAd as well as insights into the mechanism of action of EnAd and the ability of the virus to gain access to and replicate in tumours following systemic dosing.