DCE-MRI assessment of vascular changes induced with bevacizumab with or without interferon-α2a in advanced renal cell carcinoma

Introduction

Two phase-3 clinical trials, AVOREN (Escudier et al. 2007) and CALBG 90206 (Rini et al. 2008) established the benefit of the combination of bevacizumab and interferon-α2a in the treatment of advanced renal cell carcinoma. With both trials lacking bevacizumab only arm, the additional benefit conferred to bevacizumab treatment by interferon-α2a is therefore unknown, as is whether anti-angiogenic effects mediate any of this benefit.

This phase 2 study was designed to assess whether the addition of Interferon-α2a to bevacizumab increased the effect of bevacizumab in tumour vasculature in the treatment of metastatic / locally advanced renal cell carcinoma as measured by the changes in the vascular parameters of Dynamic Contrast Enhanced MRI (DCE-MRI).

Objectives

Primary Objectives

- To establish whether bevacizumab induced changes in DCE MRI vascular parameters are significantly enhanced by interferon-α2a.
- And if so to establish whether there is an IFN dose response in potentiating bevacizumab induced changes in DCE-MRI vascular parameters.

Primary endpoint to assess the primary objective was defined as the DCE-MRI defined changes in K^{trans} after 6 weeks of bevacizumab monotherapy or bevacizumab and low or standard dose IFN-α2a.

Secondary Objectives

- To correlate changes in DCE-MRI vascular parameters for each treatment group with the following:
    - progression free survival
    - tumour response
    - other surrogate biomarkers

- To assess the degree of change in baseline K^{trans} within each arm of treatment.
- To investigate changes in Diffusion and BOLD MRI and their correlation with other pharmacodynamic endpoints.

- To assess the efficacy and safety profile of bevacizumab monotherapy or in combination with low or standard doses of IFN

Study design and assessments

This clinical trial was designed as a three-arm randomised multi-centric open-labelled phase 2 study. Metastatic (stage IV) or locally advanced (inoperable stage III) RCC with good or intermediate prognosis by Motzer score (Motzer, Bander et al. 1996, Motzer, Bacik et al. 2002) who were systemic treatment naïve in metastatic setting formed the subjects of this trial.

Recruitment was planned from three UK centres - Mount Vernon Hospital, The Royal Marsden Hospital and Addenbrooke's Hospital. Eligible patients were randomised to one of the three treatment arms (10 patients per arm), with treatment regimens as below:

- Arm A: Bevacizumab 10mg/kg every 2 weeks
- Arm B: Bevacizumab 10mg/kg every 2 weeks plus low-dose IFN-α2a 3MU Three times in a week (t.i.w), commencing on Day 0.
- Arm C: Bevacizumab 10mg/kg every 2 weeks plus standard dose IFN-α2a 9 MU t.i.w. Patients will commence on IFN-α 3MU t.i.w on Day 0, and escalate dose to 9MU t.i.w on Day 14.

All patients continued on their randomised treatment regimen until the first tumour assessment at week 8. At this point the decision to introduce or modify the interferon-α2a dosage was made at the discretion of the treating physician. Bevacizumab dosing remained unchanged.

Patients in all arms underwent two baseline DCE-MRI scans in the week pre-treatment and then further scans at weeks 2 (prior to IFN dose escalation in Arm C), and 6 post-commencement of bevacizumab. Two baseline DCE-MRI were performed to assess the reproducibility of these imaging parameters. Patients also underwent CT scans of the chest, abdomen and pelvis performed at baseline, at week 8 and three-monthly thereafter. This is standard practice in our institution and hence patients were not exposed to extra CT scans and unnecessary radiation. Tumour response was to be assessed by RECIST criteria as per standard practice.

Recruitment and randomization to arms

Of the three sites open for recruitment, only two sites recruited patients for the trial. The first Centre was opened for recruitment in September 2009, and the first patient recruited in December 2009. 25 patients were screened for the study between December 2009 and

February 2012. As the recruitment was very slow, the steering committee decided to perform an un-planned interim analysis after 24 months of recruitment to assess for any trends to justify continued recruitment in the trial. At this point, excluding the 4 screen failures, 21 patients were randomised into three arms. Analysis of the primary endpoint data (change in K^{trans}) showed no trend in difference between the arms and the decision was taken that it would be futile to complete the planned accrual of a further 10 patients.

6 patients received bevacizumab only, 6 patients had bevacizumab in combination with standard dose (9MU) IFN and 9 patients were randomised to bevacizumab and low dose IFN (3MU). One patient in the bevacizumab monotherapy arm was taken off the trial before the week 8 primary end point analyses and hence was replaced as per protocol. All remaining 20 patients completed the primary end point. All patients had metastatic disease with no prior systemic treatment. 6 patients had the primary renal tumour in-situ.

At the time of data collection, all 20 patients had progressed on treatment and had been taken off-study. A total of 5 patients had to be excluded from the primary DCE-MRI analysis due to technical reasons. These included movement and cardiac artefacts, technical failure of MRI to load, breath-hold failure and one where the lesion in the chest wall was too small to characterise with DCE-MRI. 15 evaluable patients and hence 60 MRI (2 pre-treatments and two on treatment per patient) scans were thus analysed: Four patients in the bevacizumab monotherapy arm, four receiving bevacizumab and standard dose IFN (9MU) and 7 patients treated with bevacizumab and 3MU IFN.

Safety analysis

A planned independent safety analysis was performed after 15 patients completed their primary end point on the study. All adverse events including clinical and laboratory AE were

collected and graded according to CTCAE version 3.0. Causality of the AE were determined and the data obtained was analysed by an independent expert.

G1-G2 Adverse Events

With bevacizumab monotherapy (Arm A), 4 patients (80%) developed AEs. 14 adverse events were reported in this group with an average of 2.8 AEs per patient. In Arm B, where patients had bevacizumab and 3MU of interferon-α2a, all 5 patients (100%) had low grade adverse events. This group had 45 adverse events reported so on average each patient developed 9 AEs. All 5 patients in Arm C (100%) developed AE with 41 adverse events in total. On average each patient developed 8.2 AEs.

G3-G4 Adverse Events

Grade 3/4 AE were noted in 1 of the 5 patients treated with bevacizumab monotherapy (20%). On addition of low dose of IFN, 3 of the 5 patients (60%) developed high-grade side effects with a total of 4 high grade AEs.

When high dose interferon-α2a was used (Arm C), interestingly only 20% i.e. 1 of the 5 patients developed a higher grade AE.

SAEs

Six (40%) of the 15 patients had a SAE. Only 1 of the 6 SAE reported was treatment-related which required intervention. No deaths due to adverse events were reported.

1) Pt 1 – Bladder obstruction caused by clot retention unrelated to study drug
2) Pt 7 – Per rectal bleed assessed as not related to Avastin although drug discontinued
3) Pt 10 – Hospital admission due to Chest infection
4) Pt 12 – Episatxis Grade 2 – related to Avastin required cauterisation
5) Pt 19 – Pregnant partner notification
6) Pt 16 – Hospitalisation due to viral illness.

Primary variable analysis (K^{trans})

Aim of the primary analysis was to assess the changes in DCE-MRI defined parameter K^{trans} from baseline to week 6 on treatment between the three arms of the trial. To assess the true change in K^{trans} due to treatment, baseline reproducibility was initially calculated. After normalising and excluding any outliers, these values as percentage for individual patients were noted to be 33.26 for K^{trans}, 18.12 for k_{ep}, 11.25 for v_e and 17.18 for $IAUGC_{60}$.

The mean decrease in K^{trans} for all patients was -2.25% at week2 and -11.33% at week 6. Analysing treatment cohorts, between baseline and week 6, Arm A had a decrease in K^{trans} of 26.44% (95%CI -17.42 to -35.46). Changes in K^{trans} in Arm B and C were -4.56% and 32.84% (SD of 35.09) respectively. Changes in K^{trans} at week 6 (compared to baseline) was statistically significant for Arms A and C but not for Arm B. No statistical significance or trend were noted in the observed change between treatment arms.

Secondary variable analyses

Secondary analyses included assessment of other DCE-MRI associated vascular endpoints, efficacy analyses, safety endpoints and exploratory assessments including laboratory endothelial cells quantification.

Secondary MRI variable analyses (Vascular end point)

At week 2 comparison was made in the changes of K^{trans} from baseline between bevacizumab alone group (Arm A) vs bevacizumab + interferon-α2a groups (Arms B + Arm C). Arm A showed a change of -0.03% in K^{trans} from baseline and combined IFN groups

showed a change in K^{trans} of -1.360 with a standard deviation of 33.06. These values were also not considered to represent a true i.e. statistically significant change.

Similar calculations were performed for all the other MRI parameters including k^{ep}, v_e and $IAUGC_{60}$. Changes in all MRI parameters from baseline to week 2 and week 6 are reported in Table 1.1

Table 1.1 Change of MRI parameters at different time points in trial.

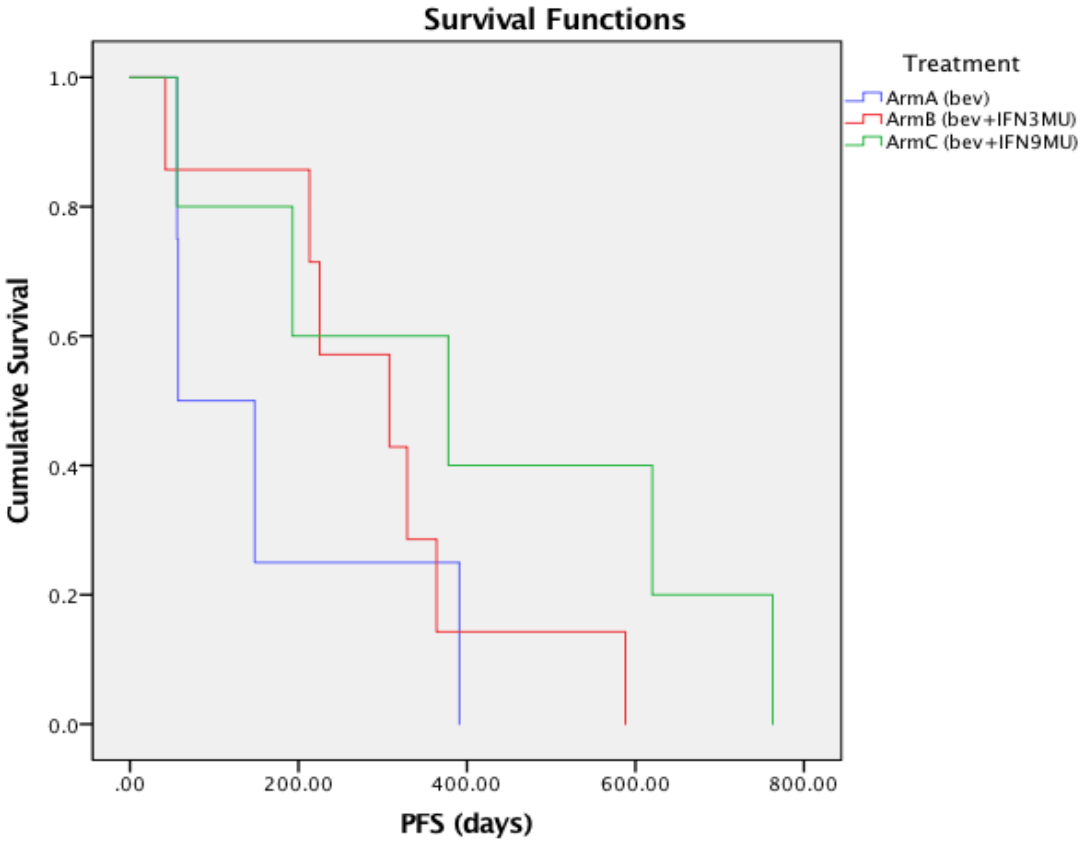
	baseline (mean)	Week2 (change in %)	Week6 (change in %)
Arm A (bev)			
K^{trans}	0.3445	-0.03	-26.439
k_{ep}	1.022	-2.877	-9.017
v_e	0.319	3.015	-10.559
$IAUGC_{60}$	28.11	9.952	-10.593
Arm B (bev +3MU IFN)			
K^{trans}	0.3997	18.12	-4.56
k_{ep}	1.358	-2.808	-11.947
v_e	0.304	17.684	7.213
$IAUGC_{60}$	34.99	8.506	-6.114
Arm C (bev+9MU IFN)			
K^{trans}	0.3726	-35.45	-32.841
k_{ep}	1.997	-15.111	-20.08
v_e	0.1865	-21.369	-17.181
$IAUGC_{60}$	24.648	-16.043	-29.646
Combined ArmB+C			
K^{trans}	0.3885	-1.36	
k_{ep}	1.59	-7.282	
v_e	0.261	3.482	
$IAUGC_{60}$	31.229	-1.313	

No statistically significant changes in secondary outcome measurements was seen. A trend of decrease of the Kep values was noted between the arms at week 6 was seen (p=0.862 Arm A vs B and p=0.450 Arm A vs C).

Clinical outcome analysis (efficacy end points)

With clinical outcome analysis, median progression-free survival (PFS) of all evaluable patients was 308 days. With cohort analysis comparing the PFS, Kaplan-Meier statistics were used and a log-rank test used to assess the significance. Median PFS for Arm A was 108 days (95% CI 0 -147). PFS was 308 days (95% CI -95.0 - 520.9) for Arm B and 378 days (95% CI 0 – 775) for Arm C. No significance was noted with p value obtained by log-rank test. Kaplan-Meier curve for PFS is shown in Figure 1.1

Figure 1.1 Kaplan Meier curve comparison of PFS between the treatment arms.



Log-rank test was performed for overall analysis and paired comparison between two arms each. χ^2 test was performed and p value calculated from the log-rank test. This is detailed in

Table 1.2. Log-rank test showed no significance between any of the treatment arms in Kaplan-Meier analysis.

Table 1.2 Log-rank test: Significance of PFS difference between treatment arms

Pairwise Comparisons (PFS vs Treatment arms)							
	Treatment	ArmA		ArmB		ArmC	
		χ^2	p-value	χ^2	p-value	χ^2	p-value
Log-rank (Mantel-Cox)	ArmA			.573	.449	1.669	.196
	ArmB	.573	.449			1.589	.208
	ArmC	1.669	.196	1.589	.208		

Comparison of PFS with MRI parameters

PFS was plotted against the vascular parameters of K^{trans}, k_{ep}, v_e and $IAUGC_{60}$ and Spearman Correlation coefficient "r" was calculated to assess any trend for correlation (Table 1.3)

Table 1.3 Spearman correlation coefficient comparing the vascular parameters and progression-free survival

Spearman correlation coefficient (rho): MRI parameters vs PFS			
	Baseline	Percentage Change of MRI parameter in Week2	Percentage Change of MRI parameter in Week6
K^{trans}	0.165 (0.557)	-0.391 (0.167)	-0.279 (0.313)
k_{ep}	0.287 (0.33)	-0.585* (0.028)	-0.47 (0.077)
$IAUGC_{60}$	-0.165 (0.557)	-0.359 (0.228)	-0.111 (0.693)
v_e	-0.0276 (0.319)	-0.166 (0.572)	0.043 (0.879)

Rho for two tailed test according to Spearman's-coefficient with alpha 0.05. Values in parenthesis suggests significance of the correlation (Zar 1972).

No significant statistically correlation was noted between PFS and K^{trans} values at baseline or the changes in K^{trans} during treatment. Subsequent analysis performed similar analysis for all

MRI parameters reviewed here including k_{ep}, v_e and $IAUGC_{60}$ measured at baseline, week 2 and week 6. Correlation was noted with change of kep at Week 2 and PFS but this was not sustained in the change of kep at week 6.

Clinical benefit

The aim of this analysis was to assess whether MRI vascular parameters at baseline, week 2 or week 6 could predict and identify patients who get any meaningful clinical benefit with the treatment. We felt that with the median progression free survival of 10.5 months with the combination treatment of bevacizumab + interferon-α2a as reported by the trials, (Escudier, Bellmunt et al. 2010), the patients could be categorised into two groups with a PFS cut-off of 6 months.

Patients who had PFS ≤ 6months and >6months were re-categorised from the trial data. Baseline and mean change in the parameters were assessed and Mann-Whitney U test was used to assess the significance of difference between the groups (table 1.4a and 1.4b)

Table 1.4a Comparison of vascular parameters (K^{trans}, k_{ep}) with clinical benefit (PFS).

	K^{trans}			k_{ep}		
	Baseline	Change at Wk2	Change at wk6	Baseline	Change at Wk2	Change at wk6
Group 1 PFS <6m	0.349	12.18	0.959	1.302	13.62	9.41
Group2 PFS >6m	0.3802	-3.274	-17.479	1.507	-10.63	-17.21
p value	0.662	0.423	0.582	0.756	0.033	0.197

Table 1.4b Comparison of vascular parameters (v_e & IAUGC) with clinical benefit (PFS)

	v_e			$IAUGC_{60}$		
Group 1 PFS <6m	0.301	-0.88	-10.88	30.59	2.01	-11.94
Group2 PFS >6m	0.264	5.75	-0.6	30.7	0.97	-14.41
p value	0.6672	0.596	0.756	0.857	*	0.952

There was no statistically significant difference in MRI vascular parameters between the groups of patients who derived clinical benefit of >6months compared to those who did not. Greater changes in K^{trans} and k_{ep} were seen in patients with PFS > 6 months however due to the small sample size these changes were not statistically significant. Even though changes in k_{ep} value from baseline to week 2 showed a p value of 0.033 between the two groups suggesting a statistical significance, this was not sustained in the changes noted at week 6 and with the small sample size (n=5, group 1) this is unlikely to represent a true change.

Discussion.

This trial attempted to address whether IFN measurably adds to the anti-angiogenic effect of bevacizumab. The AVOREN and CALBG 90206 trials (Escudier, Pluzanska et al. 2007), (Rini, Halabi et al. 2008) both compared combination bevacizumab and interferon with single agent interferon. Both studies lacked a single agent bevacizumab arm. Data regarding the efficacy of single agent bevacizumab treatment in metastatic RCC is therefore limited to that available from small phase 2 trials. The additional benefit conferred to bevacizumab treatment by IFN is therefore unknown, as is whether any benefit is mediated by anti-angiogenic effects.

The trial accrued very slowly. Many patients with metastatic RCC did not have lesions of a size or location that enabled DCE-MRI analysis. Additionally, competing first line studies and the inability to access sunitinib in the NHS at some Centres after patients had been treated with bevacizumab on trial resulted in the very slow recruitment and one centre being unable to recruit any patients.

As evident from the MRI analysis, even among patients with lesions suggested to be suitable for analysis, 5 (25%) patients were unable to be analysed due to technical issues including movement artefacts despite attempts to correct motion, cardiac artefacts, technical failure of MRI to load and breath hold failure.

Among the patients analysed, no correlation was found between change in K^{trans} and addition of IFN to bevacizumab. Effect size analysis was performed due to the smaller sample size recruited and the change in K^{trans} was still not noted to be significant.

Change in K^{trans} and k_{ep} may identify a group of patients likely to have PFS > 6 months (p 0.03), but this observation needs to be tested in a larger sample size. The small sample size makes it difficult in this case to analyse the significance of this change.

Although K^{trans} is the best-studied DCE-MRI parameter, the importance of k_{ep} or rate constant, which measures the efflux of the contrast from the extravascular extracellular space is increasingly recognised. Pre-clinical studies in a mouse xenograft model have suggested that k_{ep} might be a better parameter in assessing response to anti-angiogenic agents (Song, Cho et al. 2013). This need to be evaluated in a larger cohort.

In summary, in this small study we were unable to demonstrate significant differences in vascular parameter change between RCC patients receiving single agent bevacizumab or combination bevacizumab and IFN. The study was technically demanding and patients were difficult to accrue. Change in K^{trans} and k_{ep} may identify patients likely to have more durable benefit from anti-angiogenic treatment but this observation needs to be replicated in a larger sample size.