EudraCT Clinical Trial Results: Primary Endpoint (Assessment of Immune Infiltration [CD8$^+$ Effector T Cells])

EudraCT number	2014-004388-20
Protocol number	CRUKD/15/004
Protocol title	A Cancer Research UK randomised, double-blind, placebo-controlled Phase IIa trial of AMG 319 given orally as a neoadjuvant therapy in patients with human papillomavirus (HPV) positive and negative head and neck squamous cell carcinoma (HNSCC)
Sponsor	Cancer Research UK, Centre for Drug Development 407 St John Street, London, United Kingdom, EC1V 4AD
End of Trial date	03 May 2018

For the purpose of posting clinical trial results for the Cancer Research UK clinical trial CRUKD/15/004 to the European Clinical Trials Database (EudraCT), the following text, tables and figures summarising the analysis of immune infiltration (CD8$^+$ effector T cells) from the trial has been extracted from the approved Clinical Study Report (Version 1.0, dated 09 April 2019):

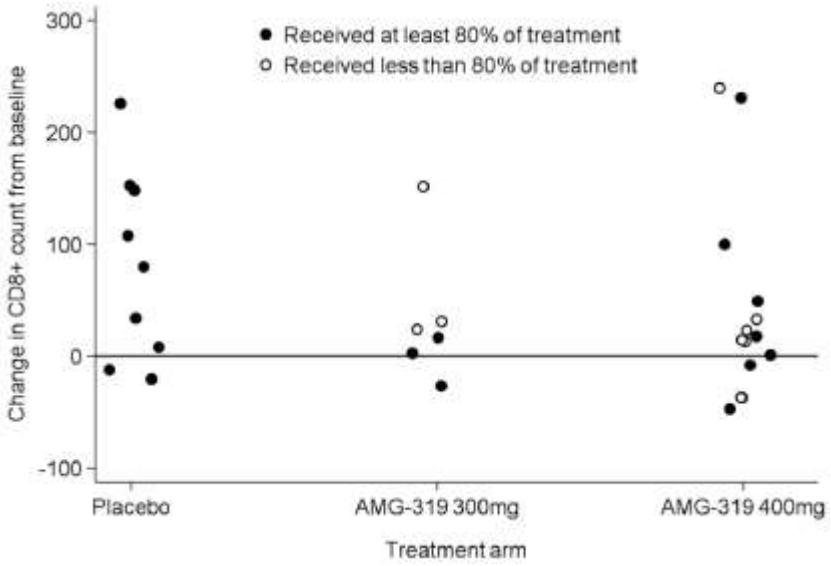
Primary endpoint: Assessment of Immune Infiltration (CD8$^+$ Effector T Cells)

Tumour tissue samples for assessment of CD8$^+$ effector T cells were collected. Pre- and post-treatment tissue samples were analysed from 30 patients and results reported for 59 sections. No tumour was present in the post treatment tissue sample for one patient and so results for this patient are not included in this efficacy analysis. Nineteen patients received at least 80% of planned IMP doses and had pre and post treatment tumour tissue available for assessment of immune infiltration by immunohistochemistry and were included in the Per Protocol (PP) population analysis.

Tissue sections were analysed by two independent and blinded pathologists who quantitated immune cell numbers (CD8$^+$ effector T cells) per high power field (hpf) for assessment of immune infiltration. The aim was to analyse 10 random hpfs [×800] per section, however some (33) of the sections obtained contained a limited area of tumour and so fewer hpfs were examined in these samples.

Changes in the CD8$^+$ count from baseline were calculated and are shown in Figure 1. Results of the primary analysis of CD8$^+$ count at resection are presented in Table 1.

Figure 1 Scatterplot Showing Change in CD8⁺ Count from Baseline by Treatment Arm



Efficacy outcomes were to be analysed separately for Human Papilloma Virus (HPV) negative and positive patients. In the models that used all available samples and considered CD8⁺ count at resection as the outcome, there was no evidence of an interaction between HPV status and treatment arm (p=0.18), i.e. the treatment effect of AMG 319 compared to placebo was not influenced by HPV status. Furthermore, in these models there was no interaction between HPV status and AMG 319 dose (p=0.07), i.e. no association between the effect of AMG 319 dose and HPV status. As there were no patients who received at least 80% of their planned AMG 319 doses who were also HPV positive, it was not possible to test for an interaction between HPV status and treatment arm in the PP population.

Table 1 Results of Linear Regression Analysis on CD8⁺ Count at Resection

Model[1]	Comparison	Change in log ($CD8^+_{resection}$)[2]	95% Confidence Interval (CI)	P-value
All available samples for patients who received AMG 319 or placebo (n=29)				
Unadjusted	AMG 319 vs Placebo	-0.23	-0.82 to 0.36	0.43
Adjusted for HPV status	AMG 319 vs Placebo	-0.22	-0.80 to 0.37	0.45
Unadjusted	AMG 319 300 mg vs Placebo	-0.40	-1.18 to 0.39	0.31
	AMG 319 400 mg vs Placebo	-0.16	-0.79 to 0.48	0.62
Adjusted for HPV status	AMG 319 300 mg vs Placebo	-0.44	-1.21 to 0.33	0.25
	AMG 319 400 mg vs Placebo	-0.11	-0.74 to 0.52	0.71
PP population (n=19)				
Unadjusted	AMG 319 vs Placebo	-0.31	-1.02 to 0.39	0.36
Adjusted for HPV status	AMG 319 vs Placebo	-0.22	-0.95 to 0.50	0.52
Unadjusted	AMG 319 300 mg vs Placebo	-0.87	-1.88 to 0.14	0.09
	AMG 319 400 mg vs Placebo	-0.09	-0.83 to 0.66	0.81
Adjusted for HPV status	AMG 319 300 mg vs Placebo	-0.80	-1.80 to 0.21	0.11
	AMG 319 400 mg vs Placebo	0.02	-0.74 to 0.78	0.96

[1] *All models allow for the baseline value of CD8⁺ for each patient.*

[2] *If the 'change in log(CD8⁺)' is zero, this indicates no difference between the two trial arms.*

The change in $CD8^+$ count from baseline (value at resection minus value at baseline) was calculated and analysed for each patient. In the PP population (n=19) the mean change in $CD8^+$ count in patients who received placebo was 80.42 (standard deviation [sd] of 84.95) whereas the mean change in $CD8^+$ count in patients who received AMG 319 was 33.75 (sd of 80.21). The standardised difference observed in this population was -0.57 (95% confidence interval -1.48 to 0.36, t-test p value=0.23, non-parametric Mann-Whitney test p value=0.22) compared to a target standardised difference of +0.50.

In the models that used all available samples (n=29), the mean change in $CD8^+$ count in patients who received placebo was 80.42 (sd of 84.95) whereas the mean change in $CD8^+$ count in patients who received AMG 319 was 39.73 (sd of 80.94). The standardised difference observed in this population was -0.50 (95% confidence interval -1.23 to 0.31, t-test p value=0.23, Mann-Whitney test p value=0.22) compared to a target standardised difference of +0.50; in this trial, placebo patients had a greater increase in $CD8^+$ count.

The ratio of post/pre $CD8^+$ T cell counts (fold increase in $CD8^+$ count from baseline) are presented in Figure 2. Results of the linear regression analysis performed are provided in Table 2.

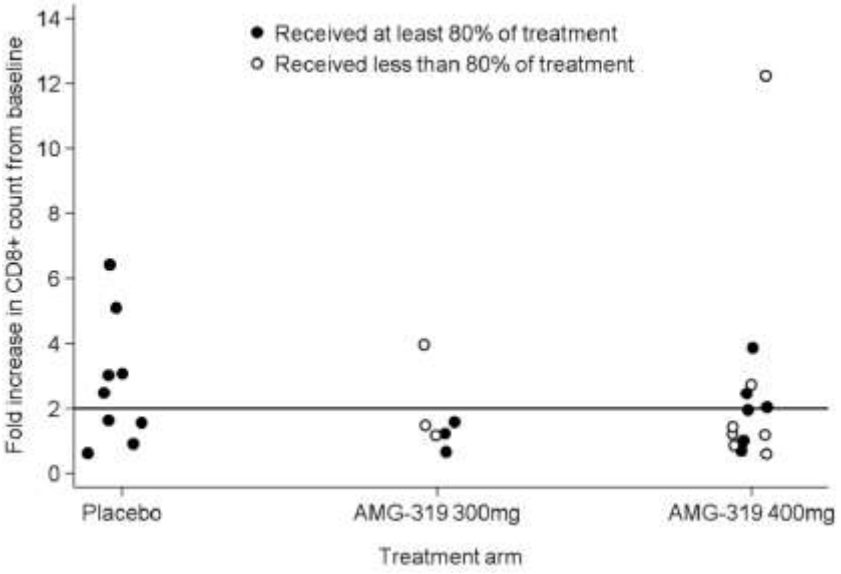
In the models that used all available samples and considered the ratio of $CD8^+$ count at surgical resection to $CD8^+$ count at baseline as the outcome, placebo patients in general experienced a greater increase in $CD8^+$ count compared to those who received AMG 319 although the wide confidence intervals include the no effect value (change in $\log[CD8^+_{resection}]$ for AMG 319 versus placebo [adjusted for HPV status] of -0.27, 95% CI -0.81 to 0.26, p=0.31). There also was also no evidence of an interaction between HPV status and treatment arm (p=0.88) or HPV status and treatment dose (p=0.85). Again, it was not possible to test for an interaction in the models that used the restricted subset of patients.

Table 2 Results of Linear Regression Analysis on Ratio of Resection to Baseline $CD8^+$Count

Model*	Comparison	Change in $\log(CD8^+_{resection}/CD8^+_{baseline})$	95% CI	P-value
All available samples (n=29)				
Unadjusted	AMG 319 vs Placebo	-0.29	-0.84 to 0.27	0.29
Adjusted for HPV status	AMG 319 vs Placebo	-0.27	-0.81 to 0.26	0.31
Unadjusted	AMG 319 300mg vs Placebo	-0.45	-1.19 to 0.28	0.22
	AMG 319 400mg vs Placebo	-0.22	-0.82 to 0.38	0.46
Adjusted for HPV status	AMG 319 300mg vs Placebo	-0.51	-1.21 to 0.20	0.15
	AMG 319 400mg vs Placebo	-0.16	-0.74 to 0.41	0.56
Only patients who received at least 80% of planned trial treatment (n=19)				
Unadjusted	AMG 319 vs Placebo	-0.46	-1.07 to 0.16	0.14
Adjusted for HPV status	AMG 319 vs Placebo	-0.39	-1.03 to 0.25	0.22
Unadjusted	AMG 319 300mg vs Placebo	-0.90	-1.79 to 0.00	0.05
	AMG 319 400mg vs Placebo	-0.27	-0.93 to 0.38	0.39
Adjusted for HPV status	AMG 319 300mg vs Placebo	-0.84	-1.75 to 0.06	0.07
	AMG 319 400mg vs Placebo	-0.20	-0.88 to 0.49	0.55

** All models adjusted for $CD8^+_{baseline}$.*

Figure 2 Scatterplot Showing Fold Increase of $CD8^+$ Count from Baseline by Treatment Arm



The number (percentage) of patients who showed doubling of $CD8^+$ T cell counts were examined between treatment groups using a Fisher's exact test. In the PP population, eight of 19 patients (42.1%) experienced a doubling of the $CD8^+$ count; five of these patients received placebo and three received AMG 319 400 mg (Fisher's exact test p=0.37). In the population that used all available samples, 11 of 29 patients (37.9%) experienced a doubling of the $CD8^+$ count; five of these patients received placebo and six received AMG 319 (five patients received AMG 319 400 mg and one patient received AMG 319 300 mg), (Fisher's exact test p=0.24).