Aflibercept efficacy according to sidedness, RAS and BRAF mutations. Findings from the VELOUR trial in second line therapy of advanced colorectal cancer patients

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ABSTRACT

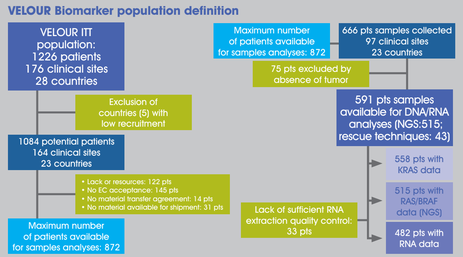
- ***Background:*** Addition of (ziv)-Aflibercept (A) to FOLFIRI in second-line therapy for metastatic colorectal cancer (mCRC) has been shown to be beneficial in phase III VELOUR trial (NCT00561470). A retrospective follow-up study (NCT01754272) was undertaken to acquire tumor samples for biomarker analyses and identify subgroups of pts with differential treatment effects. The primary results assessing efficacy according to well-established mCRC subgroups defined by RAS, BRAF status and sidedness are reported here.
- ***Material and methods:*** Tissue specimens were collected for 666 pts from 1226 ITT pts. Suitable specimens were assayed for somatic mutation using NGS targeting extended RAS and BRAF genes. NGS assays with no missing values were obtained for 482 pts. Affymetrix gene chip technology was used for whole-transcriptome profiling; sidedness was extracted from available pathological reports. Differences between subgroups were assessed by interaction analysis.
- ***Results:*** The treatment effects on overall survival (OS) for the 482 pts is still significant HR=0.80 (CI 0.65-0.99), and similar to the ITT (n=1226) results (HR=0.82, CI 0.71-0.93). Two established ways of defining mutations (traditional KRAS exon 2 and extended RAS using NGS) show reduced treatment efficacy in mutants. Interestingly, BRAF mutants (which are all RAS wild types) show a trend of better outcome response. Same is seen for PFS and RR. Sidedness did not affect efficacy (HR: 0.83 (0.63-1.1) for left and HR: 0.83 (0.54-1.3) for right).
- ***Conclusion:*** This is the only study that evaluated the impact of RAS, BRAF and sidedness of an anti-angiogenetic drug in the second line of mCRC. Lack of significant interaction between subgroups show that Aflibercept efficacy is not impaired by RAS mutations or sidedness. However, Aflibercept seems to have a specific effect on BRAF mutated tumors. *Sanofi supported this ISS. Clinical trial information: NCT01754272*

INTRODUCTION AND METHODS

- The phase 3 VELOUR trial showed that Aflibercept added to FOLFIRI significantly improved overall survival (OS), PFS and ORR compared with FOLFIRI alone (median OS: 13.50 vs 12.06 months (HR=0.817; CI 95%: 0.713-0.937; p=0.0032); median PFS: 6.90 vs 4.67 months (HR=0.758; CI 95%: 0.661-0.869; p=0.00007); ORR: 19.8% vs 11.1% (p<0.001)) in mCRC patients previously treated with an oxaliplatin-containing regimen.[1]
- A follow-up non-interventional study (NCT01754272) was undertaken to acquire archived tumor materials, with the purpose of identifying subgroups of patients with differential treatment effects.
- Methods: Archived tissue specimens (FFPE blocks) from participating centers world-wide were collected in a centralized pathology facility. Specimens with suitable quality and quantity were assayed using next-generation sequencing (NGS) targeted at KRAS, NRAS and BRAF genes. Based on the mutations, the patients were classified into four groups (with no overlap): KRAS exon 2 ("KRASex2"), other KRAS and NRAS mutations ("newRAS"), BRAF V600E ("BRAF") and double wild-type absent in all aforementioned RAS or BRAF mutations ("WT").
- Affymetrix gene chip technology was used for whole-transcriptome profiling. Here we present the results of two signatures: the "BRAF-like" and "RAS-like" signatures, that were derived on external datasets and applied to VELOUR. Patients are dichotomized into "mutant-like" or "wild-type-like", based on cutoff that puts 55% of the patient as "KRAS-mutant-like" and 7% "BRAF-mutant-like", based on the frequencies of respective mutations in DNA data.

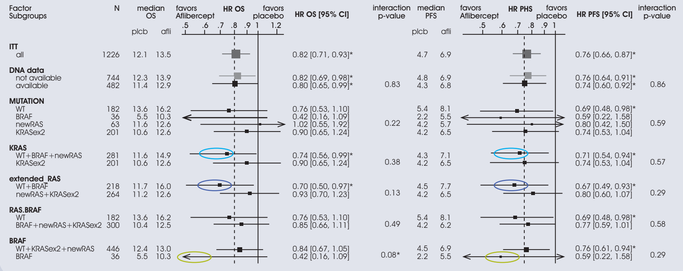
Biblio: 1. Van Cutsem E et al. J Clin Oncol. 2012 Oct 1;30(28):3499-506

VELOUR Biomarker population definition



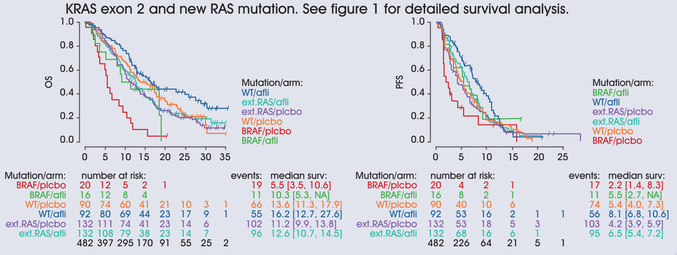
- The treatment effects on overall survival for the biomarker subpopulation (n=482, or 39.3% of original ITT, n=1226) is still significant HR=0.80 (CI 0.65-0.99), p=0.043, and similar to the ITT results (HR=0.82, CI 0.71-0.93, p=0.0031). Pretreatment rates with Avastin and patient demographics are similar except for fewer samples from America in the biomarker group.
- **Figure 1** shows summary results of treatment effects on OS under various subgroupings. The treatment effect of the original trial is shown on the first line (ITT:all). The second line shows the summaries of the subset where DNA data are available versus those that are not, showing that the biomarker subset has representative baseline HR. The subsequent subdivisions ("mutation", "KRAS" and so on) are all pertaining to the those with DNA available. The vertical dashed line is aligned with the HR of the samples with available DNA.

Figure 1 Treatment effects on OS and PFS, subgrouped by DNA mutations. (*) Indicates significant p-value (0.05 for HR, 0.1 for interaction). Cox regression models stratified by ECOG status and prior bevacizumab were used to obtain HR and interaction tests.



- In the comparison of the four mutation classes, we see the trend of WT and BRAF mut having better HR than the total, while newRAS and KRASex2 are worse, as also reflected by the differences in the median survival. However, the confidence intervals are wide (none of the subgroups are significant on its own) and the global interaction test is also not significant (p=0.22) **(Figure 2).**

Figure 2 Kaplan-Meier curves for OS and PFS, sub-grouped by DNA mutations. `ext.RAS´ is a pool of KRAS exon 2 and new RAS mutation. See figure 1 for detailed survival analysis.



CONCLUSIONS

- This retrospective biomarker analyses tested RAS and BRAF defined patient subgroups for differential treatment effects.
- Data suggest that Aflibercept could have a specific beneficial effect in *BRAF* mutant patients. This is the first randomized trial with an antiangiogenic drug to show a BRAF biomarker/drug interaction. *BRAF*mut pts numbers remain small and these results need further validation in similar trials to inform clinical practice.
- RAS defined subgroups show no significant interaction, although the ratios of treatment HR favor RAS wild types and appear to be specifically strong. Similar trends were observed in published trials with bevacizumab or ramucirumab for KRAS mut and merit further investigation.
- Aflibercept appears to result in comparable benefit regardless of primary tumor location.

ACKNOWLEDGEMENTS

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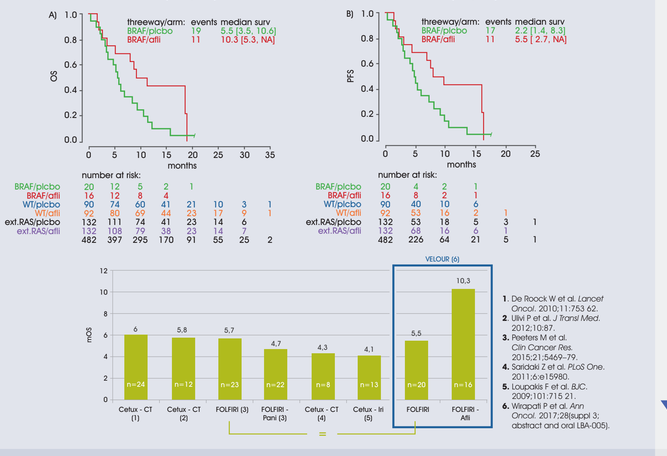
Displayed data have been presented previously at either ASCO (Chicago June 2-6, 2017) or WCGI (Barcelona June 28 - July 1, 2017) congresses.

RESULTS

- Pairwise comparisons for several ways to define mutation groups also show no significant interaction except for BRAF mutant versus others for OS. The treatment HR's for BRAF are not significant due to small number, but the magnitude and direction are consistent.. to those of other studies. The Kaplan-Meier curves in **Figure 3** highlight the treatment effects of Aflibercept on BRAF mutants.

Figure 3 Kaplan-Meier curves for OS and PFS in "BRAF", RAS Mutant and "WT" **(a)**. Median OS in BRAF Mutant 2nd-Line mCRC: control arm in line with previously published trials **(b)**



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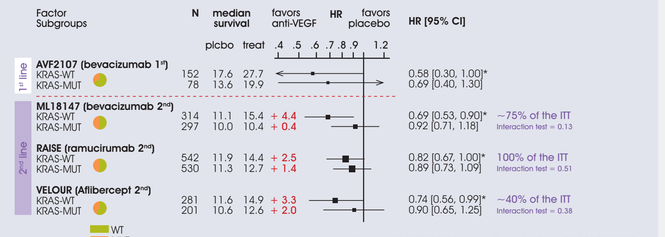
- For RAS mutants, there is a consistent trend of quantitative reduction of effects, although the interaction tests are not significant. The ratio of OS hazard ratios (RoHR) of mutant relative to wild-type for KRASex2 is 1.21 (95%CI: 0.74 – 1.96; p=0.45), and 1.39 (95%CI: 0.90 – 2.13; p=0.13) for extRAS respectively, indicating a potential loss of 20 to 40% of the positive Aflibercept effect, not reaching statistical significance. Similar studies have been reported for KRAS exon2 and other antiangiogenic drugs, summarized in **Figure 4.**

Figure 4 Summary of published KRAS biomarker studies and VELOUR data



Ince W, et al. JNCI. 2005;97:981-9; Kubicka S et al. *Ann Oncol.* 2013;24:2342-2349; Obermannova et al. *Ann Oncol.* 2016 ;27:2082-2090.

- Aflibercept seems to be as effective on right and left side in terms of OS and PFS. For OS: left HR = 0.86 [0.64 – 0.15], right HR = 0.85 (0.53 -1.35), interaction p-value = 0.96. For PFS: left HR = 0.74 (0.46 – 1.00), right HR = 0.70 (0.42 – 1.15), interaction p-value = 0.69. (**Table 1**)

Table 1 Left versus right side origin of tumors has no effect on Aflibercept efficacy

	OS	PFS
Left	HR 0.86 (0.64-1.15)	0.74 (0.46-1.00)
	Interaction p-value=0.96	
Right	HR 0.85 (0.53-1.35)	0.70 (0.42-1.15)
	Interaction p-value=0.69	