MMP-9 as predictive factor for response and progression free survival in breast cancer patients treated with bevacizumab and pegylated liposomal doxorubicin.

A multicenter, single-arm phase II trial (SAKK24/06). On behalf of the Swiss Group for Clinical Cancer Research (SAKK).

The Swiss Oncology Research Network

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Number: P2-16-07

Background

The benefit of bevacizumab (Bv) has been shown in different tumors including colorectal cancer, renal cancer, pulmonary non-small cell cancer and also breast cancer. However to date, there is no established test evaluating the angiogenic status of a patient and monitoring the effects of anti-angiogenic treatments.

Tumor angiogenesis is the result of a balance between multiple pro- and anti-angiogenic molecules. There is very little published clinical data exploring the impact of the anti-angiogenic therapy on the different angiogenesis-related molecules and the potential role of these molecules as prognostic or predictive factors.

We measured prospectively the levels of 6 angiogenesis-related molecules in the peripheral blood of breast cancer patients treated with a combination of Bv and pegylated liposomal doxorubicin (PLD):

- **VEGF:** Vascular endothelial growth factor is a potent pro-angiogenic factor. VEGF was shown to be related to breast cancer stage, prognosis and possibly response to treatment.
- **VEGFR-2** (KDR/Flk-1)**:** VEGF-receptor-2 is the main receptor inducing VEGF-response. sVEGFR-2 = soluble VEGFR-2.
- **VEGFR-1** (Flt-1)**:** VEGF-receptor-1 is considered to be an endogenous negative regulator of angiogenesis by binding and inactivating circulating VEGF. However its function is poorly understood. sVEGFR-1 = soluble VEGFR-1.
- **VEGFR-3** (Flt-4)**:** VEGF-receptor-3 is involved in lymphangiogenesis, but is also expressed on endothelial cells of angiogenic blood vessels. sVEGFR-3= soluble VEGFR-3.
- **MMP-9:** Matrix metalloproteinase 9 plays a critical role in tissue remodeling during development in pathological processes. Functional interdependence was described between VEGF and MMP-9[2].
- **PIGF:** Placenta growth factor promotes migration of endothelial cells for neo-angiogenesis and metastasis. PIGF is a ligand of VEGFR-1.

Objective

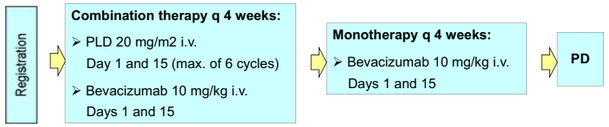
The objective of this substudy is to identify surrogate markers of angiogenesis in advanced breast cancer patients treated with the combination of PLD and Bv.

Methods & Materials

Main eligibility criteria for patients included in the SAKK 24/06 trial:

- Cytologically or histologically proven breast cancer, either metastatic or locally recurrent inoperable and HER2-negative
- Measurable disease according to RECIST
- Normal heart function (LVEF ≥ 55%) and WHO performance status 0 or 1
- No previous chemotherapy for metastatic or inoperable locally recurrent breast cancer; no previous adjuvant or neo-adjuvant chemotherapy within 12 months before registration

Treatment schedule



Blood sampling:

- 20 ml of blood were taken: 10 ml for serum and 10 ml for plasma (EDTA-K)
- The samples were processed rapidly and frozen at -80°C (transient conservation at -20°C was allowed)

Time points:

1. Baseline (before first trial treatment administration)
2. After 2 cycles of treatment (2 months)
3. Every 3 months until progression or trial treatment interruption
4. At treatment discontinuation

Measurement of the angiogenesis-related molecules:

- Enzyme-linked immunosorbent assays (Quantikine, R&D Systems and Reliatech) were used to measure the molecules
- The measurements were done centrally in our laboratory (CePO and ISREC, Epalinges, Switzerland).

Statistical considerations:

For the measure of circulating angiogenesis-related factors, all analyses were descriptive and exploratory. Hence, p-values were not corrected for multiple testing and thus considered statistically significant for $p \leq 0.05$.

The log-transformed data (to reduce the skewness) for each marker was analyzed using an analysis of variance (ANOVA) model to determine if there was a difference between the mean of the subgroups of interest, (CR+PR vs PD, CR+PR+SD vs PD) where $\alpha = 0.05$. The untransformed data was also analyzed in the same manner as a sensitivity check.

Box-plots of the log-transformed data of the main molecules categorized into the subgroups of interest were produced. Cox proportional hazards regression models were also investigated using the baseline levels as a covariate for each molecule.

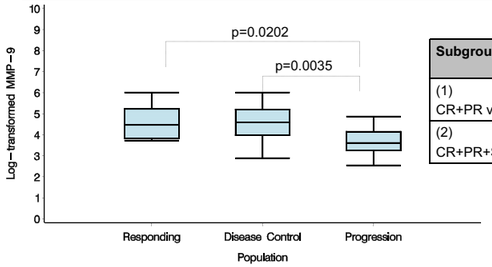
Results

- A total number of 43 patients were included in the SAKK 24/06 trial.
- 41 patients participated in this translational substudy across 11 Swiss centers.
- A total of 132 samples were collected.

Relationship between baseline levels and treatment effect

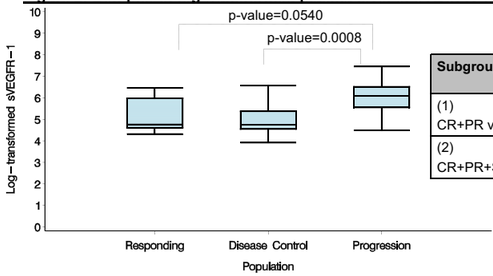
An ANOVA was used to determine if there was a difference in the means between the subgroups of interest.

Figure 1: Box-plot of log-transformed plasma level of MMP-9 for best response*



Subgroup	Log-fold change
(1) CR+PR vs PD	0.8786
(2) CR+PR+SD vs PD	0.8427

Figure 2: Box-plot of log-transformed plasma level of sVEGFR-1 for best response*



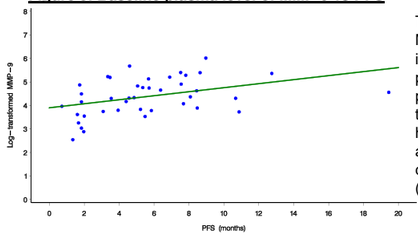
Subgroup	Log-fold change
(1) CR+PR vs PD	-0.8160
(2) CR+PR+SD vs PD	-1.0289

*Responding = CR or PR, disease control = CR + PR + stable disease, progression = progression as defined by RECIST criteria v1.0

There were no statistically significant differences in the log-transformed levels of the other angiogenesis-related molecules (VEGF, VEGFR-2, VEGFR-3 or PlGF) between the subgroups of interest.

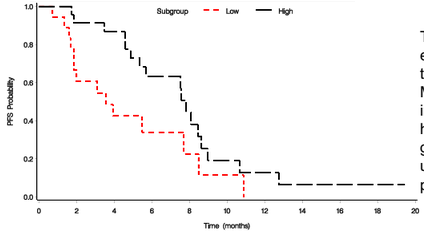
Relationship between baseline levels and outcome

Figure 3: Baseline plasma level of MMP-9 vs PFS



The log-transformed level of MMP-9 at baseline was identified as a significant prognostic factor using a Cox proportional hazards model in terms of PFS: p=0.0417, hazard ratio (HR)=0.574 with a corresponding 95% confidence interval (0.336 - 0.979).

Figure 4: Survival function estimates vs PFS



The survivor function estimates were obtained for the log-transformed level of MMP-9 at baseline grouped into low (>= median) and high (> median). These two groups were compared using a log-rank test: p=0.0408.

Conclusion

Our exploratory results suggest that:

1- The baseline plasma level of MMP-9 was associated with tumor response and disease control to PLD-Bv.

2- The baseline plasma level of MMP-9 could predict PFS.

3- The baseline plasma level of sVEGFR-1 was associated with disease control.

Therefore, these results justify further assessment of MMP-9 and sVEGFR-1 as predictive or prognostic factors in breast cancer patients treated with anti-angiogenic therapies.

References:

1. Rochlitz et al., Ann Oncol 2010; Advance Access published July 1
2. Zaman et al., Int.J.Cancer 2006; 118:755

Acknowledgments:

Swiss Federation against Cancer (Oncosuisse), the Swiss State Secretariat for Education and Research (SER) and Roche Switzerland for their support.

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Poster presented at the 33rd Annual San Antonio Breast Cancer Symposium (SABCS). San Antonio, Texas; December 2010