Liquid Biopsy in Ovarian Cancer (OC)

LOH, circulating small non-coding RNAs, Mesothelin, L1CAM, Afamin, CA 125, EpCAM autoantibodies, PD-L1, PD-L2, HLA-g.

We also focused on **blood-based biomarkers** in **OC** and analyzed **ctDNA** in blood serum of our patients. In this context, we showed that loss of heterozygosity (**LOH**) at the polymorphic microsatellite marker **D6S1581 before surgery** was **predictive** for a **reduced PFS** and **OS (Figure 1; Kuhlmann et al., 2012)**. **LOH at D10S1765 after chemotherapy** was significantly associated with the presence of **DTCs** which was consistent with our previous LOH investigations in primary OC tumor tissue (**Figure 2; Kuhlmann et al., 2011**).

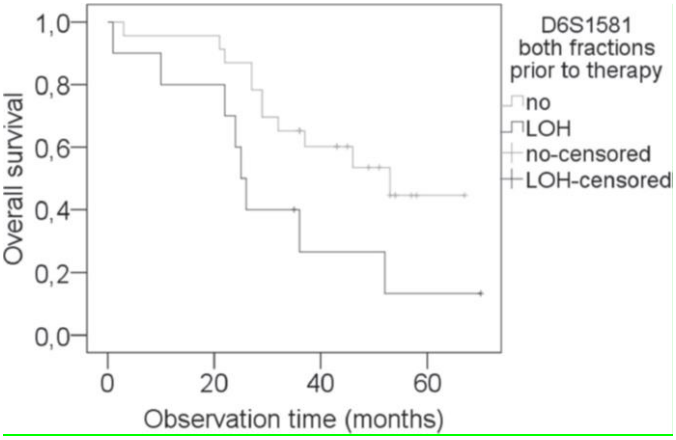

Figure 1: Correlation of LOH at D6S1581 and OS. Kaplan-Meier curves depict OS analysis of patients with and without LOH incidence at marker D6S1581 in a combined analysis of both fractions, before surgery. Top curves, patients with retention of the two alleles at D6S1581. Bottom curves, patients with LOH at marker D6S1581 (***Kuhlmann et al., 2012***).

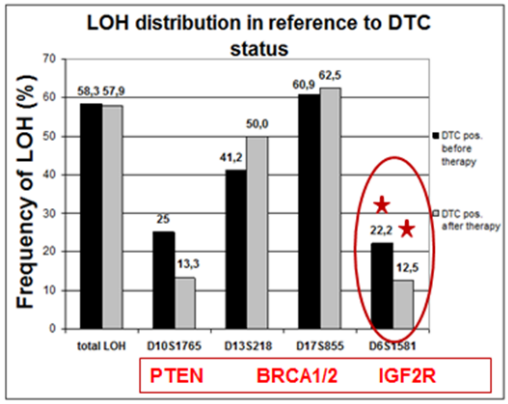

Figure 2: LOH distribution in relation to DTC status. The LOH frequencies at four selected microsatellite markers according to the presence of DTC before surgery (black bars) and after chemotherapy (gray bars) is shown. Statistical significances of these comparative analyses, as determined by the Mann-Whitney-t test, are indicated (***Kuhlmann et al., 2011***).

Furthermore, we showed **for the first time** that fragments of a **circulating small non-coding RNA**, referred to as **U2** small nuclear RNA (RNU2-1f), were **significantly elevated** in blood and documented that **persistent RNU2-1f positivity** indicated **poor prognosis**. For patients with suboptimal debulking, preoperative RNU2-1f concentration was associated with radiographic response after chemotherapy and with platinum resistance (**Figure 3**). Interestingly, according to the RNU2-1f abundance dynamics, **persistent RNU2-1f positivity before surgery** and **after chemotherapy** identified a subgroup of patients with **high risk of recurrence** and **poor prognosis** (**Figure 4; Kuhlmann et al., 2014**).

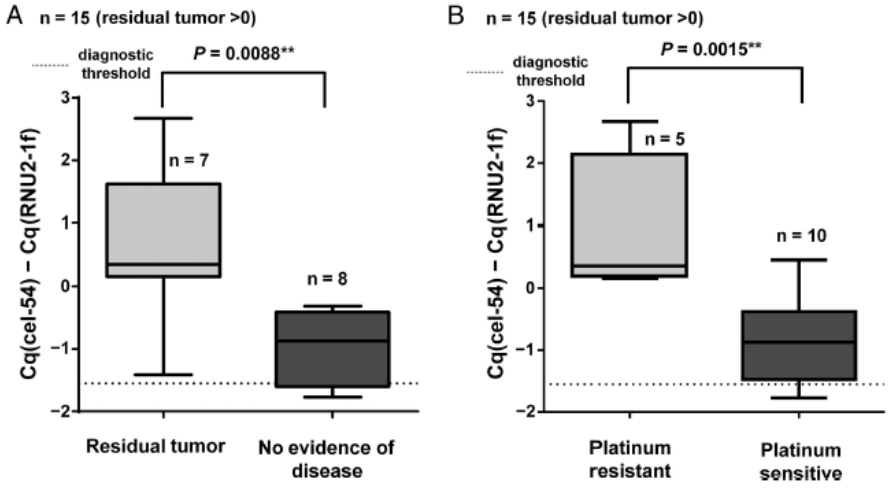

Figure 3: Correlation of RNU2-1f concentration with the patient's radiographic response and platinum resistance. (A). The box plot depicts circulating RNU2-1f abundance for patients with residual abdominal tumor after chemotherapy, as detected by abdominal CT or PET-CT scanning versus patients with no evidence of disease. (B), RNU2-1f concentration of platinum-resistant patients versus platinum-sensitive patients. **, Very significant (***Kuhlmann et al., 2014***).

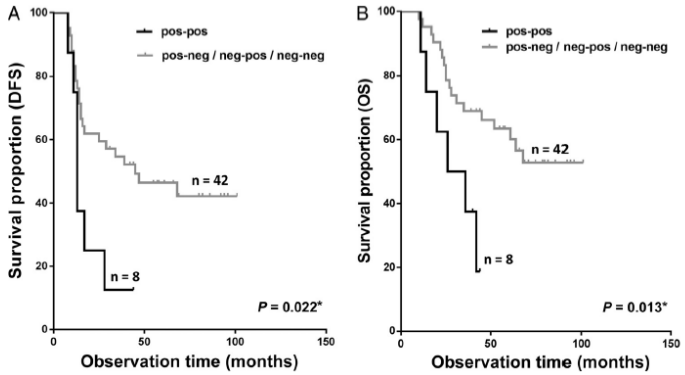

Figure 4: Correlation of RNU2-1f abundance dynamics with the patient's survival. Kaplan–Meier analyses depict prognostic significance of RNU2-1f abundance dynamics in regard to the patient's PFS (A) and OS (B). Survival curves of the persistently RNU2-1f–positive subgroup (pos-pos) were compared with all other groups together (pos-neg/neg-pos/neg-neg). *, Significant (***Kuhlmann et al., 2014***).

Other liquid biopsy markers included the analysis of **Mesothelin, L1CAM, Afamin, CA 125 and EpCAM autoantibodies**.
Before surgery, **mesothelin** positivity significantly correlated with **advanced FIGO stage, residual postoperative tumor, serous histological subtype** and **higher age**. Elevated **CA125 levels** significantly correlated with advanced **FIGO** stage and **grading**. After chemotherapy, Mesothelin as well as CA125 levels, were significantly associated with FIGO stage and residual tumor while **L1CAM** correlated with **platinum sensitivity** (**Figure 5; Aktas et al., 2013**).

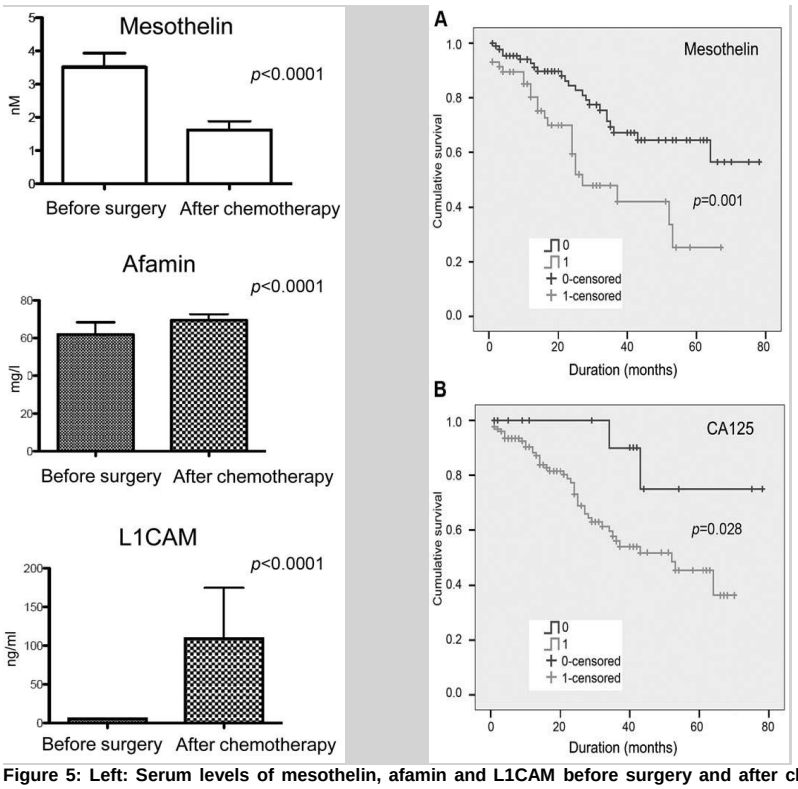

Figure 5: Left: Serum levels of mesothelin, afamin and L1CAM before surgery and after chemotherapy. Concentrations are depictetd in means. **Right: Relationship between marker expression and clinical outcome (*Aktas et al., 2013*).**

We further evaluated whether **EpCAM-autoantibodies** (AABs) have an impact on the clinical course of OC patients and demonstrated a significant **post-therapeutic increase** of AABs which correlated with **tumor resection status** after first-line therapy. Analysis of PFS, survival, FIGO stage, grading, age and sensitivity to platinum-based chemotherapy did not reveal significant associations with EpCAM-AAB titers (**Figure 6; Heubner et al., 2011**).

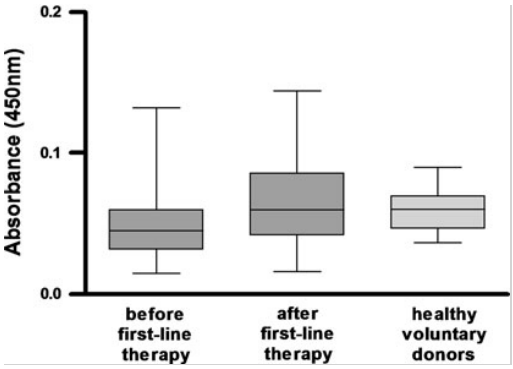

Figure 6: ELISA revealed significantly different mean AAB-levels between HCs and patients before and after first-line treatment (P<0.0001, Kruskal–Wallis Test) (*Heubner et al., 2011*).

Immunological markers

Since ligands of the Programmed Death Receptor-1 (**PD-L1 and PD-L2**) play a crucial role within the tumor microenvironment for tumorigenesis, we investigated levels of sPD-L1 and sPD-L2 in liquid biopsies of serum samples, and correlated the results with the clinical status, presence of **CTCs** and **disease outcome** in primary OC patients.

sPD-L1 was significantly **increased** and **sPD-L2 decreased** in OC patients compared to controls (**Figure 7**). While enhanced **sPD-L1** was associated with **residual tumor burden**, reduced **sPD-L2** levels were related to **platinum-resistance** (**Figure 8**) and the presence of ***ERCC1*+ CTCs (Figure 9)**. High **sPD-L1** levels were associated with a **reduced five year OS** and **PFS**. Strikingly, **sPD-L1** levels **>6.4 pg/ml** were indicative of a **reduced OS** and **PFS** in **platinum-sensitive patients**, while **OS** and **PFS in platinum-resistant patients** did **not differ** when patients were stratified to this cut-off (**Buderath et al., 2019**).

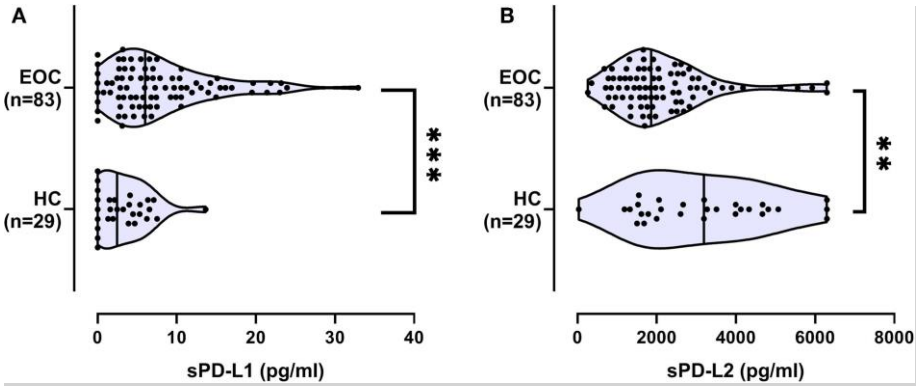

Figure 7: Serum levels of sPD-L1 (A) and sPD-L2 (B) in HCs and EOC patients. Straight line within the violin plot indicates the median. **p < 0.01, ***p < 0.001 ***(Buderath et al., 2019)***.

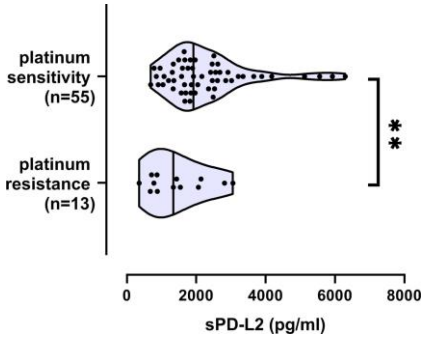

Figure 8: Decreased sPD-L2 serum levels in OC patients with platinum resistance. Straight line within the violin plot indicates the median. Platinum resistance/sensitivity was available for 68 EOC patients. **p < 0.01 ***(Buderath et al., 2019)***.

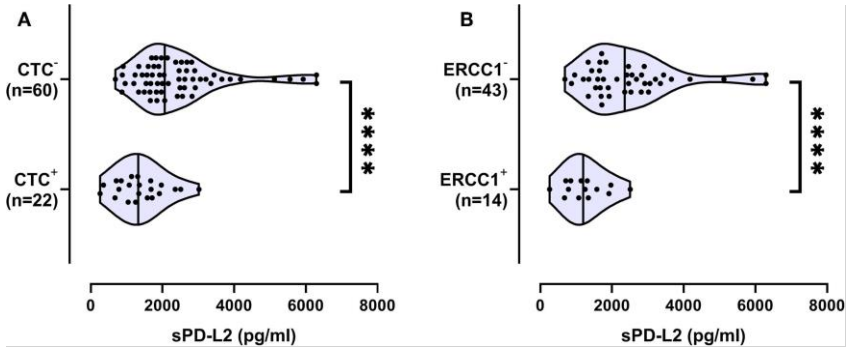

Figure 9: Association of decreased sPD-L2 serum levels (pg/ml) with the presence of CTCs and the *ERCC1*+CTC subpopulation. Data about the presence of CTC (A) or *ERCC1*+CTC (B) was available for 82 and for 57 EOC patients, respectively. Straight line within the violin plot indicates the median. ****p < 0.0001 ***(Buderath et al., 2019)***.

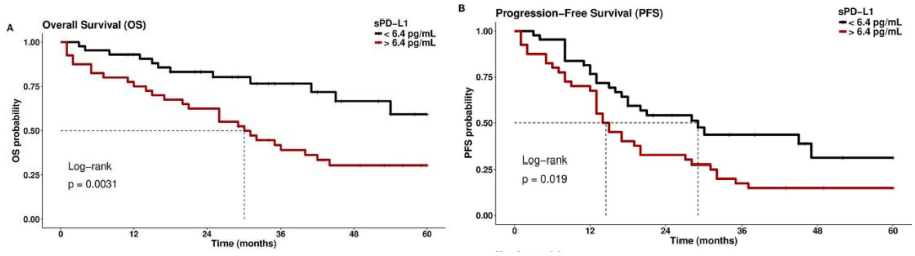

Figure 10: Kaplan-Meier curve of survival probability with respect to sPD-L1 serum levels (pg/ml). Patients with high sPD-L1 serum levels (>6.4 pg/mL) had a reduced **(A)** OS (p = 0.0031) and **(B)** (PFS; p = 0.019) compared with patients who had low sPD-L1 levels (<6.4 pg/ml). Time was calculated from blood sampling to event (death/progression) or last follows up. Dotted line indicates the median survival time of EOC patients in the respective group ***(Buderath et al., 2019)***

As the immune checkpoint molecule **HLA-G**, which is operative in immune-escape, can be **released by EVs**, we evaluate the abundance of EV and its vesicular-bound amount of HLA-G (HLA-GEV) as a biomarker in OC.

EV particle number and **HLA-GEV** were significantly **elevated** in OC patients, compared to healthy controls (**HCs**). However, elevated levels of **HLA-GEV**, but not EV numbers, were exclusively associated with a **disadvantageous clinical status/outcome**, including **residual tumor**, presence of **CTCs**, and disease **progression**. High HLA-GEV status was an independent predictor of progression, besides residual tumor burden and platinum-sensitivity. Especially among patients with no residual tumor burden or with platinum-sensitivity, **HLA-GEV identified patients** with **high risk of progression (Schwich et al., 2019)**.

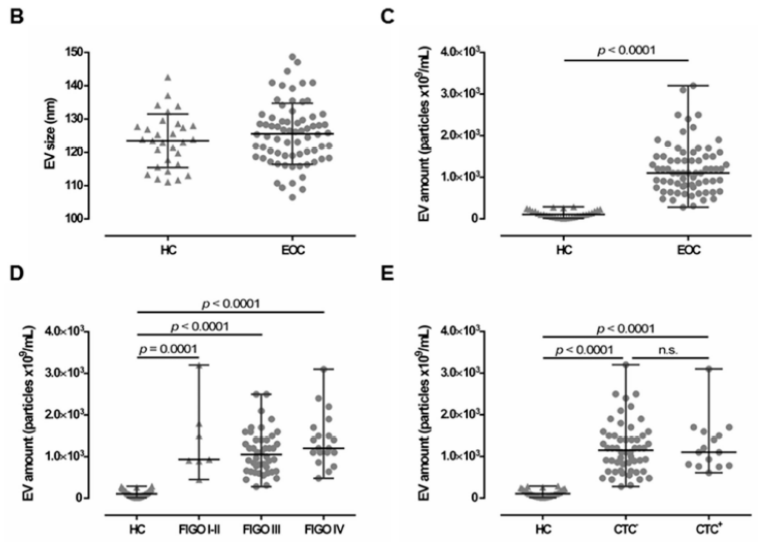

Figure 11: (B) EV size analysis revealed that EV size is similar in both, HCs and EOC patients. **(C)** The EV amount showed that EOC patients harbor significantly higher amounts of EV, compared to HC. (**D,E**) Stratification of EV amount showed enhanced EV amounts among all FIGO stages and irrespective of CTC status compared to HC. Statistical significance was tested by Mann–Whitney test (B,C) and Kruskal–Wallis test (D,E), $p < 0.05$. n.s., not significant. Triangular symbols represent HCs, while round circular symbols illustrate EOC patients (**Schwich et al., 2019**).

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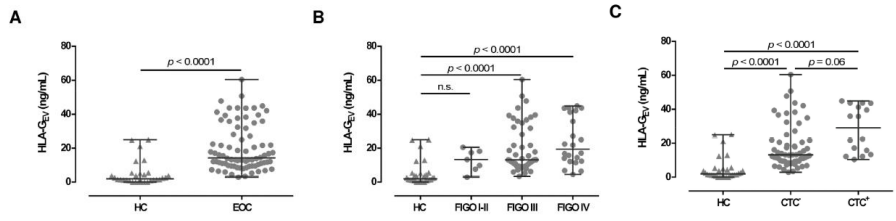

Figure 12: Association of HLA-GEV levels of EOC patients, in comparison to HC. (**A**) HLA-GEV levels are significantly increased in EOC patients, compared to HC. (**B**) Levels of HLA-GEV are increased in advanced FIGO stages. (**C**) EOC patients with detectable CTC harbor higher HLA-GEV levels, compared to HC and patients with no CTCs detected. Given is the median with range. Statistical significance was tested by Mann–Whitney test (**A**) and Kruskal–Wallis test (**B**,**C**), $p < 0.05$. n.s., not significant.Triangular symbols represent HCs, while round circular symbols illustrate EOC patients **(Schwich et al., 2019)**.

As single nucleotide polymorphisms (**SNPs**) in the **HLA-G** 3′ untranslated region (**UTR**) regulate HLA-G expression, we investigated HLA-G 3′UTR haplotypes arranged by SNPs in HCs and primary OC patients and determined soluble HLA-G (**sHLA-G**) levels.

Although **haplotype frequencies** were **similar** in patients and controls, **(i) sHLA-G** levels were **increased** in **OC** independent of the haplotype, **(ii) homozygosity** for **UTR-1** or **UTR-2** genotypes were significantly associated with **metastases** formation and presence of **CTCs** before therapy, whereas **(iii)** the **UTR-5** and **UTR-7** haplotypes were significantly associated with a **beneficial clinical outcome** regarding negative nodal status, early FIGO staging, and improved OS. Lastly, **(iv)** the ambivalent impact on clinical OC aspects could be deduced to specific SNPs in the HLA-G 3′UTR: +3187G, +3196G and +3035T alleles (**Schwich et al., 2019**).

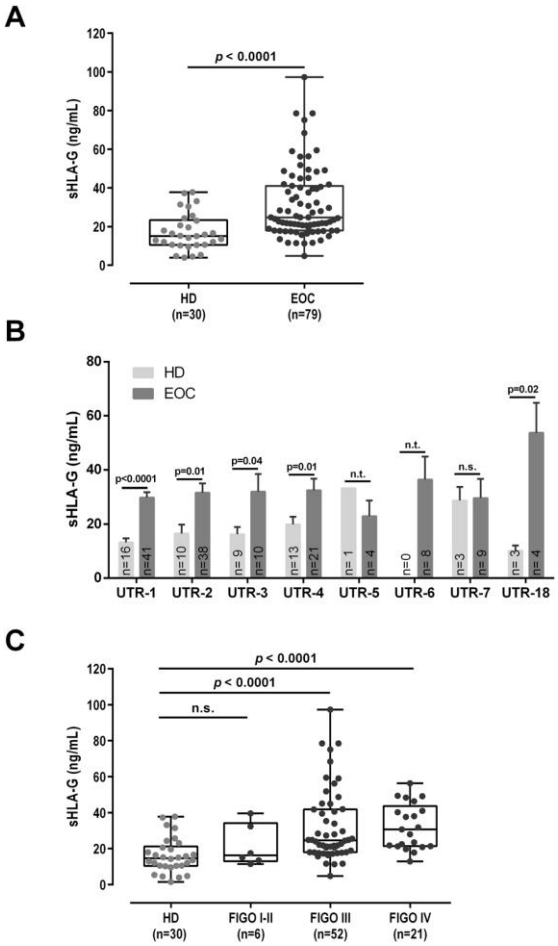

Figure 13: Comparison of sHLA-G levels of EOC patients and HCs. (**A**) sHLA-G is significantly elevated in EOC compared to HCs. (**B**) Elevated sHLA-G levels in EOC are independent of a specific *HLA-G* 3′UTR haplotype. Bars indicate mean value ± SEM. **(C)** sHLA-G levels increase with ascending FIGO stage in EOC patients without reaching significance. sHLA-G levels are given in ng/ml. Statistic was performed by Mann-Whitney test (**A**,**C**), or Kruskal-Wallis with Dunn's test for multiple comparison (**B**). n.s. not significant, n.t. not tested due to low numbers (**Schwich et al., 2019**).

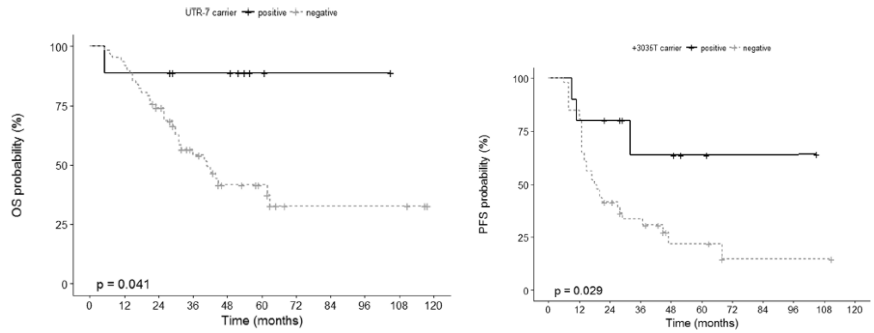

Figure 14: Association of UTR-7 haplotype of HLA-G with an improved OS and association of HLA-G 3'UTR SNP variant +3035T with an improved PFS in OC patients. (**Schwich et al., 2019**).

References

Kuhlmann JD, Schwarzenbach H, Wimberger P, Poetsch M, Kimmig R, Kasimir-Bauer S. LOH at 6q and 10q in fractionated circulating DNA of ovarian cancer patients is predictive for tumor cell spread and overall survival. BMC Cancer. 2012 Jul 31;12:325.

Kuhlmann JD, Schwarzenbach H, Otterbach F, Heubner M, Wimberger P, Worm K, Kimmig R, Kasimir-Bauer S. Primary tumor loss of heterozygosity proximal to M6P/IGF2R locus is predictive for the presence and persistence of disseminated tumor cells in the bone marrow of ovarian cancer patients. Genes Chromosomes Cancer 2011 Aug;50(8):598-605Epub 2011 May11.

Kuhlmann JD, Baraniskin A, Hahn SA, Mosel F, Bredemeier M, Wimberger P, Kimmig R, Kasimir-Bauer S. Circulating U2 small nuclear RNA fragments as a novel diagnostic tool for patients with epithelial ovarian cancer. Clin Chem. 2014 Jan;60(1):206-13.

Aktas, Kasimir-Bauer S, Wimberger P, Kimmig R, Heubner M. Utility of mesothelin, L1CAM and Afamin as biomarkers in primary ovarian cancer. Anticancer Res, 2013 Jan;33(1):329-36.

Heubner M, Errico D, Kasimir-Bauer S, Herlyn D, Kimmig R, Wimberger P. EpCAM-autoantibody levels in the course of disease of ovarian cancer patients. Med Oncol 2011 Jun; 28 (2):626-30 Epub 2010 Apr 10

Buderath P, Schwich E, Jensen C, Horn PA, Kimmig R, Kasimir-Bauer S, Rebmann V. Soluble Programmed Death Receptor Ligands sPD-L1 and sPD-L2 as Liquid Biopsy Markers for Prognosis and Platinum Response in Epithelial Ovarian Cancer. Front Oncol. 2019 Oct 15;9:1015.

Schwich E, Rebmann V, Michita RT, Rohn H, Voncken JW, Horn PA, Kimmig R, Kasimir-Bauer S, Buderath P. HLA-G 3' untranslated region variants +3187G/G, +3196G/G and +3035T define diametrical clinical status and disease outcome in epithelial ovarian cancer. Sci Rep. 2019 Apr 1;9(1):5407.

Schwich E, Rebmann V, Horn PA, Celik AA, Bade-Döding C, Kimmig R, Kasimir-Bauer S, Buderath P. Vesicular-Bound HLA-G as a Predictive Marker for Disease Progression in Epithelial Ovarian Cancer. Cancers (Basel). 2019 Aug 2;11(8):1106.