sIL-2R- an Immuno-biomarker for Prediction of Metastases in Uveal Melanoma

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Abstract. *Background/Aim:* Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. High serum levels of soluble IL-2 receptor (sIL-2R) have been reported in acute inflammations and metastatic cancers. This study evaluated the potential of high/increasing sIL-2R levels in predicting metastases. *Patients and Methods:* The study included a total of 1,546 sera samples of subjects from three groups: 119 healthy controls (73 subjects), 566 UM 10 year (10y) disease-free (DF) (220 patients), 861 metastatic UM (268 patients). Patients were followed-up biannually with liver ultrasound and liver function tests for the presence of metastases (Mets). Blood samples to measure the levels of sIL-2R were obtained at the time of primary diagnosis, soon after initial treatment (enucleation, brachytherapy), every 6 months, 10 years from diagnosis, at Mets confirmation by CT, and after additional treatments. *Results:* Significantly higher sIL-2R levels were detected in the Mets patients compared to healthy controls and 10y DF patients. Compared to the upper limit of the normal levels of sIL-2R, 1,000 U/ml, its levels in metastatic UM were 61%, 25% in 10y DF UM, and 6.25% in the controls. High levels of sIL-2R in metastatic patients, decreased significantly post treatments. Individual kinetics of markers, indicated similar trends of sIL-2R compared to osteopontin and S-Protein 100, predicting metastases, which were confirmed on liver imaging. *Conclusion:* Significantly higher sIL-2R levels were evident in all UM patients with

Mets. Significant increases in sIL-2R levels on serial evaluations indicated and predicted UM Mets, enabling earlier treatment of Mets, to improve survival.

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults with an incidence of 6-8 new cases per million people, every year. The mortality of UM due to metastases (Mets), is about 20% 5 years after diagnosis, reaching up to 50% in 15 years. The most common metastatic site of UM is the liver; in many cases it is the only affected organ. Accordingly, most ocular oncologists screen with liver function tests, and liver imaging [magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound (US)], every 6-12 months. Unfortunately, metastases are discovered at a relatively late stage with already advanced liver disease (1, 2).

Therefore, there is a need for reliable, non-invasive and low-cost tumor markers with high sensitivity, for earlier detection of Mets in UM. We have previously identified potential effective markers for UM Mets detection (3-6) such as S-Protein 100 (S-100), melanoma inhibitory activity (MIA), osteopontin (OPN), tissue polypeptide specific antigen (TPS), Vascular endothelial growth factor (VEGF), and insulin-like growth factor 1 (IGF-1). A relatively new tumor marker for UM, not published earlier, is soluble IL-2 receptor (sIL-2R). The IL-2 receptor consists of 3 subunits, and the α subunit, meaning CD25, can be cleaved and released into the blood (7).

We and others have previously reported on high levels of sIL-2R in various lymphomas and leukemia (8-14), especially in patients with higher grade disease, with good correlation to treatment responses. sIL-2R has been reported to be an extremely strong prognostic factor for patients with peripheral T-cell lymphoma unspecified (13). In hairy cell leukemia, sIL-2R has been demonstrated to be an important biomarker for its detection, disease activity, response to treatment, and prediction of early progression of disease (10).

High levels of sIL-2R have been reported in various inflammation-related conditions, especially in advanced disease stage and acute-phase of diseases such as sepsis (15), throat infections (16), ocular Behcet's disease (17), and primary biliary cirrhosis (18).

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Key Words: sIL-2R, biomarker, metastases prediction, uveal melanoma.

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Figure 1. Levels of sIL-2R (A) and percentage of sIL-2R >1,000 (B) in uveal melanoma patients and controls. Significantly higher levels of sIL-2R and percentage of sIL-2R >1,000 U/ml in metastatic and DF patients are shown, as compared to controls.

In several types of cancer, especially in the metastatic stage, high levels of sIL-2R have been reported, whereas a significant decrease has been observed after response to therapies. Furthermore, high levels of sIL-2R have been recorded in cutaneous melanoma (19), gastric cancer (20), and head and neck cancers (21, 22) compared to healthy controls.

We and others have also demonstrated that increasing levels of sIL-2R predicted metastases development and associated with a shorter survival time of patients. sIL2R has also been suggested as an independent prognosticator of Mets in many types of inflammatory diseases and cancer (19-22).

Figure 2. sIL-2R levels are significantly reduced post-treatment, as compared to those pre-treatment. (A) Effect of treatment (brachytherapy, stereotactic radiosurgery or enucleation) on sIL-2R levels in uveal melanoma patients, pre- and post-therapy. (B) Time-dependent decreases (every 2 months) in the levels of sIL-2R following treatment of individual uveal melanoma patients.

Major depressive disorder (MDD) and schizophrenia have been described to be associated with inflammatory processes. Serum levels of IL-6, IL-18, TNF α , and sIL-2R were found to be elevated in depressed and schizophrenic patients, as compared to healthy controls. A significant increase ($p<0.05$)

in serum levels of cytokines in patients with MDD and schizophrenia, in spite of lacking an overt inflammation, have been reported. Therefore, anti-inflammatory drugs as adjuvant therapy in patients with schizophrenia and MDD, have been implemented (23-27).

Figure 3. Metastases detection by sIL-2R increasing levels, in comparison to additional biomarkers: OPN and S-100, every 6 months of follow-up.

We have shown preliminary results on the role of sIL-2R in UM patients (28). This study utilized a larger study population and compared other markers that we have previously shown to be effective biomarkers for predicting metastases in UM. The aim of this study was to evaluate the potential of high or increasing sIL-2R levels to predict UM Mets development.

Patients and Methods

Blood samples were obtained at the time of primary diagnosis, soon after initial treatment (enucleation, brachytherapy), in follow-up

examinations every 6 months, at Mets confirmation, and after additional treatments for the ocular tumors. The study included a total of 1,546 serum samples of subjects from the 3 groups: 861 serum samples from Mets UM patients, 566 samples from UM patients 10y disease-free (DF) and 119 samples from healthy controls.

We included 73 healthy individuals, 220 10y DF UM patients and 268 metastatic UM patients. Patients were followed up biannually with liver imaging and liver function tests for the presence of Mets. Serum levels of sIL-2R were analyzed by ELISA tests (Abbott GmbH., Wiesbaden, Germany).

Statistical analysis was performed by using the SPSS: IBM SPSS Statistics for windows, Version 25.0, 2017. IBM Corp., Armonk, NY, USA).

Figure 4. Receiver operating characteristic (ROC) analysis of sIL-2R levels for metastases prediction (AUC=0.8139).

Results

Significantly higher sIL-2R levels were detected in the Mets patients, compared to healthy controls and 10y DF patients, as shown in Figure 1A. The study included a total of 1,546 sera samples from subjects from 3 groups: 119 samples from 73 healthy controls, 566 samples from 220 10y DF UM patients, and 861 samples from 268 metastatic UM patients. Percentage of sIL-2R sera levels >1,000 U/ml (the cut-off between normal and pathological results) in each group, were as follows: metastatic patients showed 61% higher levels, 10y DF patients- 25%, and controls - 6.25% (Figure 1B). The increasing levels of sIL-2R predicted metastases formation, which was confirmed later by liver ultrasound, CT, or MRI.

The high levels of sIL-2R in metastatic UM patients decreased significantly following treatment of the ocular tumor (brachytherapy, stereotactic radiosurgery or enucleation) every 2 months, as shown in Figure 2.

The association of sIL-2R levels with disease and response to therapy was comparable to that of OPN and S-100, which were shown formerly to be sensitive markers in UM (Figure 3A). Significant increases in the levels of sIL-2R, were very similar to those of OPN and S-100 and predicted metastases formation, as confirmed by liver ultrasound, CT and MRI, only a few months later (Figure 3B and C). Receiver operating characteristic (ROC) analysis is related in a direct and natural way to cost/benefit analysis of

diagnostic decision making. ROC analysis since 1944, has been used in medicine, radiology, and biometrics. We performed ROC analysis of sIL-2R and showed (area under the curve=0.8139), which is presented in Figure 4.

Discussion

In this study, we have clearly demonstrated that there is a strong correlation between sIL-2R levels and the stage of UM disease: low levels were found in the Controls group and in the 10y DF patients. In contrast, in the metastatic group, almost all patients had high levels of sIL-2R. Increasing levels, as shown by the kinetics of changes in the levels of the marker were associated with and predicted metastases formation. These results are very similar to those found in other diseases, as we and others have shown in inflammatory conditions, several other metastatic cancers, and in MDD (8, 10, 11, 18, 21, 27). In our former studies on UM markers, S-100 had a higher sensitivity in predicting disease than MIA and OPN by ROC analysis (3-5). In our preliminary study, as well as in this larger study, we have shown by ROC analysis that sIL-2R has a sensitivity of 81% (28). The effect of recent treatments of metastatic UM including chemotherapy, immunotherapy and new biological drugs, is questionable. Resection of isolated hepatic metastases can significantly prolong survival. However, in some cases, hepatic metastases occur in patients with normal serum enzymes (29).

Therefore, the use of blood tumor markers, which are non-invasive and highly sensitive, is a superior way to detect early metastases, as we have shown previously with the markers S-100, MIA, OPN, and TPS (3, 4). However, we have also reported on markers that are not suggested to be used in routine clinical evaluations, such as VEGF and IGF-1, due to the great variability in their levels (6, 30).

Conclusion

Significantly higher serum sIL-2R levels were found in UM patients with metastases as compared to controls. In individual serial evaluations, significant increases in sIL-2R levels, may indicate the early development of UM metastases in such patients.

Conflicts of Interest

The Authors declare no competing interests regarding this study.

Authors' Contributions

BV: Project PI and paper preparation. KI: Technical assistance. PJ: Clinical PI.

Acknowledgements

The Authors thank Shani Cohen for the technical help.

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Received January 2, 2022

Revised January 23, 2022

Accepted February 2, 2022