1. Purpose of the visit

Adrenocortical carcinomas (ACCs) are heterogeneous tumors with a poor prognosis. The rarity of this disorder causes a lack of treatment experience and material availability which is necessary to optimize existing treatments and to develop novel therapeutic strategies.
In recent years different strategies to improve transplantation models of ACC are being developed. One of the new attempted ways is the optimization of tumor models by utilizing patient material for induction of tumor xenografts in immuno-compromised mice. In fact, tumor material from patients that could be maintained in xenograft models would provide the opportunity to specifically test for the suitability of a specific compound for an individual patient, in order to achieve a personalized medicine.
Recently, Prof. Beuschlein's research group started the development and characterization of patient-individual tumor models in their laboratory and they are now transferring these models for the first time in a novel preclinical therapeutic setting for ACC, in order to test different chemotherapeutic agents and different ways of chemotherapeutic delivery.
Regarding chemotherapeutics, they are focusing their studies on liposomes and liposomal chemotherapy, since they detected an extraordinary uptake phenomenon/internalization of plain liposomes by adrenocortical tumor cells.
Thus, the exchange visit in Prof. Beuschlein's laboratory was planned with the purpose to be trained in expertise regarding in vivo studies on adrenocortical tumor models, such as NCI-H295 xenografts, but specially patient-individual tumor models.
In particular, the detailed aims of the visit were to learn:
1. the right procedure of tumor preparation for the subcutaneous implantation in athymic mice;
2. the establishment of the mouse xenograft model obtained by subcutaneous implantation of patient's tumor specimens in the animal ("personalized xenograft");
3. techniques for monitoring the implant and tumor growth as well as for analyzing tumor characteristics;
4. different methods of injection of NCI-H295 cells in the mouse models;
5. various techniques of application of different compounds (i.e. intraperitoneal and intravenous injection);
6. a method for a considerable blood sampling.
In addition, during the visit I was supposed to follow their ongoing in vivo experiment, aimed to compare the efficacy of a liposomal chemotherapy plus the adrenolytic substance mitotane versus free standard chemotherapy plus mitotane for the treatment of ACC.

2. Description of the work carried out during the visit

The training in Prof. Beuschlein's laboratory was supervised by Dr. Costanze Hantel, senior researcher in Prof. Beuschlein's laboratory and expert in mouse models.

During the exchange visit I had the opportunity to observe a subcutaneous ACC xenograft model, obtained by engraftment of small pieces of a fresh human tumor in the subcutaneous niche behind the neck of some immuno-compromised isogenic mice.
Tumors were enabled to develop and grow, then almost all the mice were sacrificed and tumors collected for histological analysis. One of the tumors, instead, underwent expansion by subsequent passage in a small cohort of isogenic mice. In brief, the tumor was retrieved from the initial xenograft and fragmented in several pieces, which were re-implanted in five mice. The final purpose will be to repeat the expansion for several passages, to achieve a large number of isogenic xenografts.

I could also follow an ongoing experiment carried out on a more commonly used adrenocortical model, that of human ACC cell line NCI-H295, which is obtained by subcutaneous injection of 6 x 10^6 NCI-H295 cells in athymic nude mice. Mice were divided into several groups, which underwent different drug treatments:

(a) etoposid, doxorubicin, cisplatin (EDP), with or without mitotane (o,p'-DDD).
(b) doxorubicin, paclitaxel, cisplatin (DPaP), with or without mitotane.

Both these drug combinations were tested in the free standard formulation or in the liposomal formulation.
Tumor growth was followedevery other dayby measuring tumor diameter by a caliper, and mice were weighted to evaluate any loss in weight due to pharmacological treatments.

Finally, during the visit they showed me their techniques of administration of different drug compounds, by intraperitoneal and intravenous injection, and their method for performing blood sampling.

3. Description of the main results obtained;

Histological analysis, performed on tumor samples explanted by mice some weeks after the subcutaneous implantation, showed that explanted tumors usually retain histological and biochemical features of the cells and tumor of origin, even if with a few exceptions, due to the high heterogeneity of the human tumors of origin.

Data obtained in a pilot short-term experiment on NCI-H295 xenograft showed that both the drug combinations tested were able to reduce tumor growth as compared to placebo treated controls. Interestingly, liposomal chemotherapy plus mitotane appeared to be more effective than the free standard chemotherapy plus mitotane. In addition, the ongoing long term experiment showed that the liposomal formulation of paclitaxel-based chemotherapy caused less adverse effects than the free standard ones (i.e. reddening, edema and crusting on skin and tails of treated mice).

4. Future collaboration with host institution (if applicable)

This exchange visit will consolidate the scientific transfer of knowledge between the Italian group of Prof. Massimo Mannelli and the German team of Prof. Beuschlein. Moreover, this was the first exchange visit between the two groups, that will be followed by a subsequent exchange visit from Prof. Beuschlein to Prof. Mannelli group in the next months. This exchange of techniques and know-how will be pivotal to homogenate research methodologies between research teams belonging to the ENSAT.

5. Projected publications / articles resulting or to result from the grant (ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant);

The know-how developed during the exchange visits planned between the two laboratories will be functional to carry out the planned projects of the two partners **(**Prof. Massimo Mannelli and Prof. Felix Beuschlein) of the ENS@T-CANCER project funded by the EU.

6. Other comments (if any).