# Treatment of Transmissible Venereal Tumors in Dogs with Intratumoral Interleukin-2 (IL-2). A Pilot Study

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**Abstract.** *Aim: To improve the treatment of transmissible venereal tumors (TVTs) in dogs with intratumoral injections of interleukin-2 (IL-2). Patients and Methods: We treated 13 dogs with 18 natural TVTs with IL-2. The tumors were treated with intratumoral application of $2\times10^6$ units IL-2. Results: Three months after injection of IL-2, the tumors in 2/13 dogs had regressed completely, those in 1/13 had regressed partially, and 4/13 dogs had stable disease. Conclusions: Local IL-2 treatment of TVT is therapeutically effective, as indicated by complete regression (CR), partial regression (PR) and stable disease (SD) of the tumors of 7 out of 13 dogs. In addition, we observed that the intratumoral treatment with IL-2 did not cause any toxic side-effects.*

Transmissible Venereal Tumors (TVTs) were first described by Nowinsky in 1876 (1). TVTs most frequently occur in dogs in tropical and sub-tropical countries. TVTs are usually transmitted from dog to dog during mating when abraded skin is exposed to the tumor of an infected dog (2, 3). Consequently, the tumors grow mainly on the genitals. The literature on TVTs is often confusing and contradictory as there are two forms of TVTs: natural and experimental TVT that, however, are not always recognized (4). Many studies focus on experimentally-transplanted or injected TVTs, which have a different disease course than naturally transmitted TVTs. This difference is probably related to differences in antigen presentation after disease transfer by contact *via* the epithelial cells or sub-epithelial cells. An experimental TVT is usually transferred by subcutaneous injection of TVT cells. After transplantation, there is usually first a progressive growth phase during 5-7 weeks followed by a static phase and a regression phase (3, 5-7). After three to nine months, the transplanted TVT is rejected by the immune system. The natural disease is transmitted by sexual intercourse or other close contact between dogs. Authors often stress that natural TVT can be transmitted because these tumor cells do not induce an immune reaction. Natural TVT does not regress spontaneously; these tumors grow or are in steady state. In immunologically incompetent or compromised hosts the tumor progresses to ulceration and metastasis (8) in about 5 % of the cases (9). This is in line with Murchison (10) and Purohit (11) who stress that spontaneous regression has not been well-documented in natural TVT.

The tumor is transmitted by viable tumor cells but not by dead tumor cells, viruses or cell-free filtrates (12). The origin of TVTs is not yet clear but, at present, most authors conclude that TVT has a histiocytic origin (3, 13-15). TVT should be differentiated from mastocytoma and malignant lymphoma (14). TVT grows as a homogeneous population of large round cells with distinctive centrally located nucleoli (3).

The infectious nature of TVT may be surprising, but TVT is not the only tumor that is spread by contact between a tumor bearer and a healthy recipient as a similar disease occurs in the Tasmanian Devil, a marsupial in Tasmania. Devil facial tumor disease (DFTD) affects the face of animals due to tumor cell transfer during fighting (10). This tumor often kills the host within 6 months (6) and is considered a threat to the endangered species.

Both DFTD and TVTs have a chromosomal abnormality (10, 14). Somatic cells in dogs have 78 chromosomes but TVTs have 57-64 chromosomes (17, 18). TVT is thought to be a single clone (14) that emerged either about 250-2,500 or 7,800-78,000 years ago (14). DFTD tumor in



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Table I. *Description of animals*.

| No. | Sex | Age | Breed | No. tu. | Tumor size 1 | Tumor size 2 | Tumor size 3 |
|---|---|---|---|---|---|---|---|
| 1 | F | 10 | Mixed | 1 | 3.5×3.0×0.7 | | |
| 2 | F | 1 | Rott-weiler | 1 | 2.2×2.2×1.1 | | |
| 3 | F | 3 | Mixed | 1 | 1.7×1.5×0.6 | | |
| 4 | F | ? | Mixed | 3 | 2.0×1.8×1.0 | 4.0×2.6×0.8 | 1.9×1.3×0.4 |
| 5 | M | ? | Mixed | 1 | 4.8×5.8×2.8 | | |
| 6 | F | ? | Mixed | 1 | 2.3×1.7×1.1 | | |
| 7 | M | ? | ? | 1 | 6.2×5.9×3.9 | | |
| 8 | M | 7 | Mixed | 1 | 6.5×5.5×2.3 | | |
| 9 | M | 7 | Mixed | 1 | 4.4×3.1×0.9 | | |
| 10 | F | ? | Mixed | 1 | 3.2×1.5×1.8 | | |
| 12 | M | 9 | Mixed | 2 | 0.8×0.4×0.2 | 1.2×1.0×0.5 | |
| 13 | F | 2 | Mixed | 1 | 5.7×2.9×1.7 | | |
| 14 | M | 2 | Mixed | 3 | 1.0×1.2×0.2 | 1.0×0.7×0.1 | 0.6x0.6x0.01 |

No., Number of animal; No. tu., number of tumors. Size in $cm^3$ (length x breadth x depth) at first presentation.

Table II. *Tumor sizes in $cm^3$ at months after injection of IL-2*.

| Dog no. | 0 month | 1 month | 3 months | 6 months |
|---|---|---|---|---|
| 1 | 7.4 | dead | | |
| 2 | 5.3 | dead | | |
| 3 | 1.5 | dead | | |
| 4 | 12.9 | 21.8 | 21.9 | 6.5 |
| 5 | 78.0 | 22.7 | 47.9 | LFF |
| 6 | 4.3 | 5.1 | 5.1 | dead |
| 7 | 142.7 | 71.6 | LFF | |
| 8 | 82.2 | 94.2 | 36.9 | 21.9 |
| 9 | 12.3 | 4.8 | 10.5 | |
| 10 | 8.6 | 11.6 | 11.0 | |
| 12 | 0.7 | 0.0 | 0.0 | |
| 13 | 28.1 | 18.5 | OOP | |
| 14 | 0.3 | 0.3 | 0.1 | 0.1 |

OOP, Out of protocol; dead, died with tumor, probably due to tumor; LFF, lost for follow-up.

Table III. *Therapeutic effect of intratumoral application of IL-2*.

| Dog no. | Months after IL-2 injection | | | Therapeutic effect |
|---|---|---|---|---|
| | 1 | 3 | 6 | |
| 1 | Dead | | | Dead |
| 2 | Dead | | | Dead |
| 3 | Dead | | | Dead |
| 4 | PD | PD | SD | PD |
| 5 | PR | SD | LFF | SD |
| 6 | SD | SD | dead | SD |
| 7 | SD | LFF | | LFF |
| 8 | SD | PR | PR | PR |
| 9 | PR | SD | LFF | SD |
| 10 | SD | SD | LFF | SD |
| 12 | ML | ML | LFF | CR |
| 13 | SD | OOP | | LFF |
| 14 | SD | PR | ML | CR |

Therapeutic effect is based on total size of all tumors in an animal. PD, Progressive disease (>150% start of therapy); SD, stable disease (50-150%), PR, partial remission (0-50%), ML, minimal lesion tissue remaining (≤0.1 $cm^3$); Dead, died with tumor; LFF, lost for follow-up: OOP, out of protocol because of change of therapeutic regime.

Tasmanian devils emerged recently, probably some decades ago as an aggressive facial tumor (10). In most cases the tumor does not metastasize but it grows and bleeds. Dogs suffering from TVTs often exude serosanguineous fluid from their TVTs , thereby infecting other dogs and soiling their living area. The predilection site of the tumor for genitals often inhibits complete surgical removal of the tumor (5). A major problem of surgery, therefore, is that most tumors recur. As such, if complete surgery is impossible, chemotherapy is considered the treatment of choice. Usually, the dogs are treated with intravenously-applied vincristine sulphate (0.5-0.7 $mg/m^2$). Intravenous application once weekly for 3-6 weeks is usually effective (6, 19). However, as it is undesirable to come into contact with spilt vincristine (especially children and pregnant women) a non-toxic replacement drug is needed.

TVTs are considered naturally-occurring allografts (20). The tumors are regarded as a consequence of the limited diversity of the major histocompatibility complex (MHC) in dogs that allow cancer cells to be transplanted from one animal to another (21). In the experimental model, different stages of tumor development are associated with different levels of MHC class I and II expression. Initially MHC levels are low but at later stages of the tumor are up-regulated. This increased MHC expression is induced by tumor-infiltrating leukocytes (22). TVT inoculation depletes dogs of their B lymphocytes (23). Because of its (transplantation) immunogenic nature, TVT has been subjected to immuno-therapy with interleukin (IL)-12 (24) and the combination of IL-6 and IL-15 (25). Although several important immune parameter values were up-regulated after these interventions, no clinically relevant results were reported.

Local IL-2 is therapeutically effective in different cancers, such as bovine ocular squamous cell carcinoma, canine mastocytoma, nasopharyngeal carcinoma and carcinoma of the bladder (28). Transplantation immunology revolves around differences in MHC class I and II. CD4+T lymphocytes recognize MHC II, CD8+ T lymphocytes recognize MHC I, natural killer (NK) cells recognize MHC I and all these cellular subsets recognized cell types that are directly activated by IL-2.

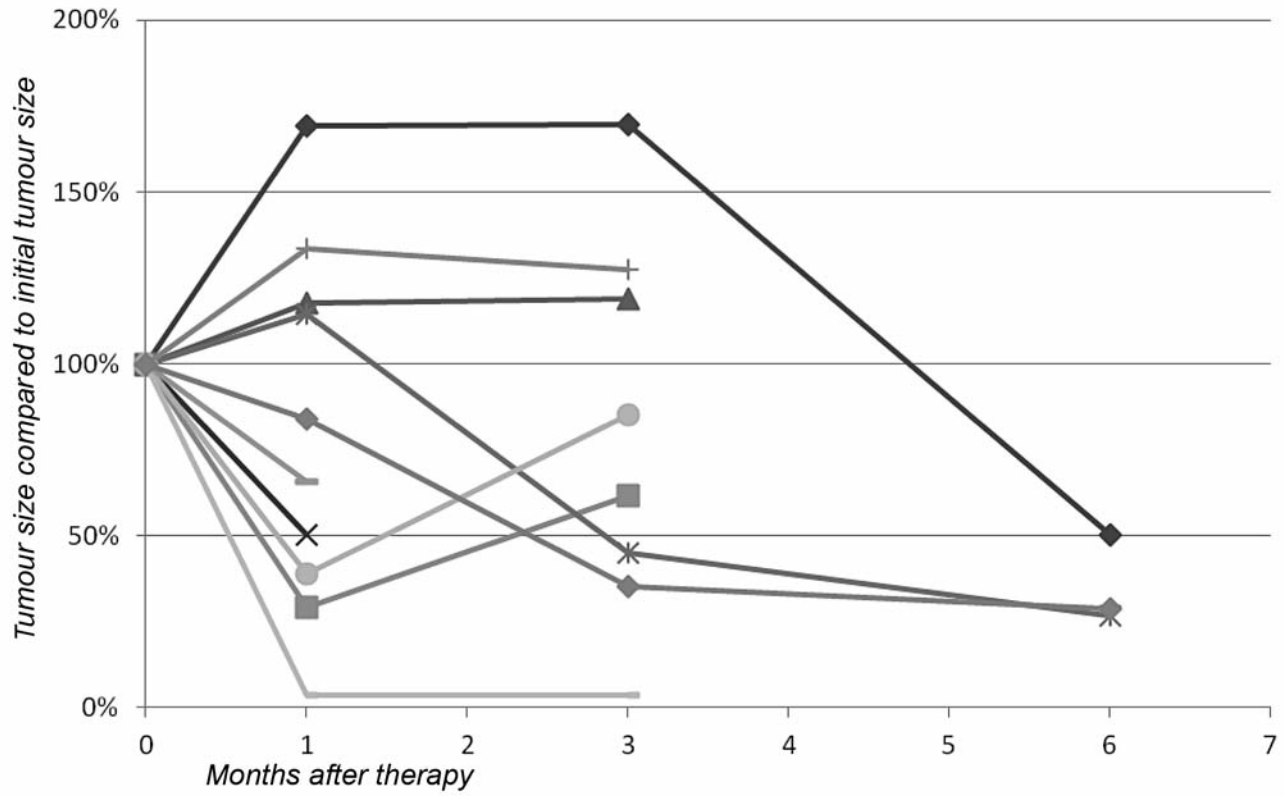


Figure 1. *Kinetics of tumor regression after a single intratumoral injection of IL-2.*

Our research, thus, focuses on the treatment of cancer with local application of IL-2. Usually, and in contrast with systemic IL-2 therapy, local IL-2 therapy is very effective (26-29). IL-2 has been used successfully for the treatment of bovine ocular squamous cell carcinoma (30-32), vulval papilloma and carcinoma complex in cattle (33), canine mastocytoma (34), human bladder carcinoma (35, 36), naso-pharyngeal carcinoma (26) and melanoma (37). Treatment of cancer with local IL-2 application is reviewed by Den Otter *et al*. (28). The present study was performed to improve the treatment of TVT with intratumoral injections of IL-2.

## Materials and Methods

*Study's subjects and treatment*. Dogs presented with TVT in the Veterinary Centre Curacao, Ronde Klip, Curacao, were included in the study. The diagnosis of TVT was histo-pathologically confirmed in all animals by the presence of the same large vacuolated tumor cells. Dogs whose housing and owners allowed for treatment with vincristine, the standard treatment for TVT, were excluded. Each patient was treated polyclinically with local IL-2 by a veterinarian (MH), with the owner's consent.

Thirteen dogs with 18 tumors were treated with intratumoral application in each lesion of $2\times10^6$ units of IL-2 (Novartis; Arnhem) suspended in 1 ml of water. The tumor was imbibed with IL-2 containing fluid. In case of very small tumors, the IL-2 suspension was not only given intratumorally but also peritumorally. The size of the tumors was measured with callipers as the product of length, width and height (volume) at 0, 1, 3 and 6 months after the first IL-2 application. The tumor volume at first presentation was compared with the volume of the treated tumors. The therapeutic effects were expressed as complete regression (CR), partial regression (PR: tumor size of 1-50 % of the original tumor), stable disease (SD: tumor size of 50-150% of the original tumor); and progressive disease (PD: tumor size of over 150% of the original tumor). Very small tumors $\leq 0.1$ cm$^3$ were considered minimal lesions (ML). We introduced ML for tumors that regress to a small tumor with a size of about 10% of the original tumor. Microscopic examination shows that, in such a small tumor usually all tumor cells have disappeared and are replaced by connective tissue. Therefore, such a small tumor is just visible/measurable macroscopically but regarded as CR when observed microscopically.

## Results

*Description of the animals*. Table I shows the results from thirteen dogs: 6 males and 7 females. There were one Rottweiler and 12 cross-bred animals. The latter suggests that most dogs were free roaming animals. Of 8 animals, the age was known, ranging from 1-10 years; with a median age of 5 years. There were 18 tumors in 13 dogs: Ten dogs had 1

tumor, 1 dog had 2 tumors and 2 dogs had three tumors. All dogs had natural (spontaneous) TVT.

Table II shows the effect of IL-2 therapy on the size of the tumor at 3 months after IL-2 application. Thirteen dogs with 18 tumors were treated. In dogs 12 and 14, bearing two and three tumors, respectively, the tumors were reduced to 0.0 and 0.1 $cm^3$, respectively (CR). Dog 8 also had a strong and lasting reduction in tumor size (PR). The remaining 4 cases of SD are also clinically relevant (Table II).

*Kinetics of tumor regression*. Figure 1 shows the kinetics of the regression of the tumors due to IL-2 therapy. In 3 dogs, it is clear that regression of a tumor is a slow process requiring months.

*The effect of therapy*. There were 2/13 CR/ML, 1 /13 PR, 4/13 SD, while 3/13 animals died and 2 tumors were lost for follow-up (Table III).

Of note, intratumorally injected IL-2 did not cause any side effects whatsoever. The cost amounted to about € 200 per complete treatment of per dog.

## Discussion

Natural TVT is in most cases caused by copulation of a TVT-diseased animal with a healthy dog. Although a transplantation reaction is expected, these tumor cells do not mount an effective immune reaction. This could be explained by the relative superficial localization of the tumor often at or near mucosal surfaces and its lack of MHC expression (22).

After treatment of 13 cases of TVT with intratumoral IL-2, two animals had a CR and another a PR. Literature shows that tumor regression is associated with influx of various types of immune cells (39, 40, 41) that are responsive to IL-2.

In a pilot study (42), we already described treatment of TVT with a single intratumoral IL-2 application; 19 dogs with TVT were treated with a single injection of $10^6$ U IL-2; 6 dogs (32%) obtained CR. In the present study, we show that local IL-2 injection in TVT affects the tumor growth as measured by comparing the size of the IL-2 treated tumor with the size of the tumor at the first presentation, expressed as CR, PR, SD, PD and ML. This approach constitutes a standard technique to measure the therapeutic effects of IL-2 therapy.

Interestingly, some researchers consider TVT as a stem cell cancer (41). Considering the relative resistance of cancer stem cells to chemotherapy and radiotherapy and their role in subsequent tumor re-growth, the present results of intratumoral IL-2 therapy might become even more interesting.

## Conclusion

The therapeutic effects after IL-2 administration were: 2/13 dogs had CR, 1/13 dogs PR, 4/13 SD, 2/13 LFF, 1/13 PD; only 3/13 died. This shows a valuable therapeutic effect in 7/13 dogs (54%). Tumor regression, due to intratumoral injection with IL-2, is often a slow process requiring months.

Intratumorally-applied IL-2 shows no toxicity whatsoever and has no leakage of toxic products. The cost amounts to 200€ per patient.

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