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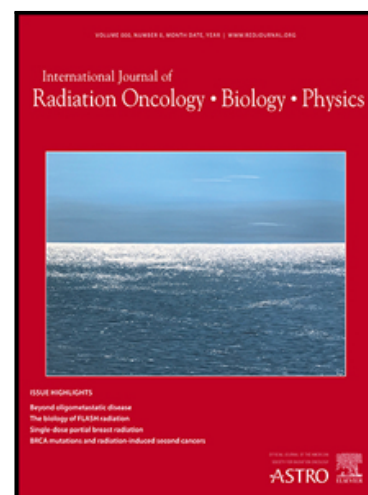
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Towards neuro-oncologic clinical trials of high dose rate synchrotron Microbeam Radiation Therapy: first treatment of a spontaneous canine brain tumor.



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Towards neuro-oncologic clinical trials of high dose rate synchrotron Microbeam Radiation Therapy: first treatment of a spontaneous canine brain tumor.

Short running title: First microbeam treatment of canine brain tumor.

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Data availability

All data generated or analyzed during this study are included in this published article.

Abstract:

Introduction: The high potential of Microbeam Radiation Therapy (MRT) in improving tumor control whilst reducing side effects has been shown by numerous preclinical studies. MRT offers a widened therapeutic window by using the periodical spatial fractionation of synchrotron generated x-rays into an array of intense parallel microbeams. MRT now enters a clinical transfer phase. As proof of principle and cornerstone for the safe clinical transfer of MRT, we conducted a “first in dog” trial under clinical conditions. In this report, we evaluated whether a 3D conformal MRT can be safely delivered as exclusive radiosurgical treatment in animal patients.

Methods: We irradiated a 17.5 kg French Bulldog for a spontaneous brain tumor (glioma suspected on MRI) with conformal high dose rate microbeam arrays (50 μ m-wide microbeams, replicated with a pitch of 400 μ m) of synchrotron-generated x-rays. The dose prescription adjusted a minimal cumulated valley dose of 2.8Gy to the PTV (CTV+1mm). Thus, each beam delivered 20-25Gy to the target as peak doses, and ~1Gy as valley doses.

Results: The treatment was successfully delivered. Clinical follow-up over 3 months showed a significant improvement of the dog's quality of life: the symptoms disappeared. MRI, performed 3 months post irradiation, revealed reduction in tumor size (-87.4%) and mass effect with normalization of the left lateral ventricle.

Conclusion: To our knowledge, this neuro-oncologic veterinary trial is the first 3D conformal synchrotron x-ray MRT treatment of a spontaneous intracranial tumor in a large animal. It is an essential last step towards the clinical transfer of MRT in the near future, to demonstrate the feasibility and safety of treating deep seated tumors using synchrotron-generated microbeams.

Keywords: synchrotron microbeam radiation therapy, glioma, translational research, veterinary trial.

Introduction:

Since the 1990's, there is a growing interest in synchrotron radiotherapy programs and numerous preclinical publications have shown the potential of these disruptive treatment modalities. The relatively low penetration of the generated X-rays in the 100keV energy range could be counterbalanced by i- the high dose rate of x-ray beams that takes advantage of the so-called flash effect (1) ii- the spatial fractionation inherent to microbeam radiotherapy (MRT (2)), pushing the limits of the dose-volume effect and iii- the option of combining synchrotron low-medium energy x-rays with high-Z radiosensitizers (3).

Preclinical studies have demonstrated the potential of MRT as it improves tumor control compared with conventional Broad Beam (BB) radiotherapy whilst reducing side effects (4–8). MRT, developed at synchrotron x-ray sources for the last 3 decades, now enters a clinical transfer phase (9). This approach widened the therapeutic window thanks to the periodical spatial fractionation of synchrotron generated x-rays into arrays of intense, quasi-parallel, 50 μ m-wide microbeams; replicated with a pitch of 200-400 μ m. Very high radiation doses (tens to hundreds of grays) are deposited in the microbeam paths (peak dose, PD) while the dose in-between microbeams (valley dose, VD) amounts to only about 5-7% of the peak dose (7). Normal brain tissue is extremely tolerant to MRT (10). Cell loss is mainly confined to microbeam paths. There is no disruption of mature vasculature, only a transient increase in vessel permeability has been measured (11). The continuous perfusion of normal tissues is maintained (6). A recent study has shown that multi-directional MRT exposures of intracranial tumors in rats leads to 2.5-fold higher tumor control probability compared with conventional radiation protocols (7) ; the low normal tissue complication probability of such MRT strategies has not only been revealed in

normal brain tissues in rodents (7), but also in minipigs (12) and developing brain of piglets (13).

To manage the complexity of MRT, medical researchers, including medical and nuclear physicists, clinicians and biologists collaborate closely with scientists and engineers at synchrotron facilities in translational research programs towards clinical trials. Before proposing MRT for clinical trials, each treatment individual step of the procedure should be validated with respect to safety standards and workflow typical for the treatment of human patients. The spontaneous tumors of veterinary patients located in depth from surface comparable to the tumors found in human patients, can be considered as excellent model for validation.

As proof of principle and cornerstone for the safe clinical transfer of MRT, we conducted the internationally first therapeutic microbeam irradiation of a spontaneous malignant brain tumor in a veterinary patient. Under the conditions of a clinical trial, we have validated the safe delivery of 3D conformal MRT as exclusive radiosurgical treatment to a pet dog bearing a malignant brain tumor

Methods and materials

Clinical case

The dog was a 6-year old male French Bulldog of 17.5kg which was presented with generalized and partial seizures. The results of clinical examination, including standard neurological tests (cranial nerve reflexes, posture, gait etc.), were unremarkable. Supportive medical treatment consisted of oral phenobarbital and prednisolone intake (5 and 0.3mg.kg⁻¹.d⁻¹, respectively). The MRI scan performed one month before treatment revealed the presence of an ovoid intra-axial mass tumor measuring 6.7cm³ located in the left temporal lobe, causing deviation of the midline and compression of the left lateral ventricle. The lesion was T₁ hypointense, T₂ hyperintense, heterogeneous on T2-FLAIR images, and partially enhanced after injection of Gd-DOTA. The epidemiological context and the clinical and imaging data suggested the presence of a glial tumor (14). The biopsy was declined by the owner.

Treatment planning and dosimetry

The animal was positioned using a patient-specific thermoplastic mask and stereotactic frame (Brainlab, Germany). A volumetric dosimetry CT scan was performed (Aquilion Lightning, Canon, Japan; 120kVp, 120mAs, 220 slices 1mm thick, 512×512 matrix, 32cm FOV), with metallic markers placed on the thermoplastic mask for the image guided MRT positioning (Figure 1A).

The treatment was planned on the Isogray workstation (Dosisoft, France). The radiation-oncologist team delineated contours (CTV, organs at risk and positioning markers, Figure 2A) on the MR images registered on the dosimetry CT. Five coplanar and conformal fields were positioned so that the beam recovery was minimized at the skin level (Figure 2).

The images, DICOM-RT plan and structures files were used for the production of Lipowitz's alloy conformal masks (Figure 1D) and the dose calculation with the hybrid dose calculation algorithm developed for MRT specificities (15). The dose prescription was realized to adjust the PTV (CTV+1mm) minimal cumulated valley dose to 2.8Gy. With these parameters, each beam would deliver 20-25Gy to the target as peak doses, and ~1Gy as valley doses.

Patient positioning, dosimetry and MRT

MRT irradiation was performed at the XXXX. The accurate positioning of the isocenter in the beam was guaranteed by a 6 degrees of freedom goniometer on which the dog was positioned in his contention mask (Figure 1B) (16). At 42m from the synchrotron source, the collimated and filtered (Be: 2.3 mm, C: 1.13mm, Al: 1.95mm, Cu: 2.08mm, Al:2mm and 0.3 μ m Au) x-ray beam had a 33mm horizontal, 0.52mm vertical size and a spectrum which extended from 50-500keV (average energy 121keV). The reference dose rate (5500Gy/s) was measured with a 2x2cm² field at 2cm depth in water (17).

The treatment isocenter was correlated on the goniometer isocenter using the image guidance method developed by Donzelli *et al.* (18) on stereoradiographic images acquired with the synchrotron beam at low flux (<50mGy/image). For the treatment, 1.3m upstream the patient, the quasi-laminar beam was collimated into an array of microbeams (50 μ m width, 400 μ m on-center pitch) using the tungsten collimator characterized in (19). The vertical scanning speed (42.8mm/s) was set to obtain a vertically extended field covering the whole PTV. Film dosimetry was performed in a solid water cube phantom, at treatment depth, according to the method described by Ocadiz *et al.* (20).

The safety of the treatment delivery was ensured by a custom-designed, redundant patient safety system that would stop the irradiation sequence within 10ms if the incoming flux suddenly changed by more than 15%.

Patient follow up

Before and after the treatment, the dog's general health and behavior were recorded by the owner using standardized calendars (to record seizure number and severity) and questionnaires. A complete general and neurological physical examination was performed by the referring veterinarian 0.5, 1, 2 and 3 months after the treatment. The dog underwent MRI control exams at 1 and 3 months after microbeam exposure, to evaluate the tumor volume.

Results

Dosimetry

The peak and valley dose (VD) maps as well as the peak and valley dose-volume histograms are presented in figure 3. Peak entrance doses were ~60Gy. The PTV received a minimum cumulated VD of 2.8Gy, an average cumulated VD of 5.1Gy and a maximum cumulated VD of 11.8Gy (respectively 27.5Gy, 109.9Gy and 196Gy for peak doses). Table 1 reports the quality assurance protocol of the calculated and measured doses per beam. Differences up to 25% were observed between measured and calculated doses. These discrepancies are still under investigation as they are undoubtedly originating from approximations in the calculations as well as from experimental bias. Indeed microbeam dosimetry remains a challenging medical physics issue due to the micrometric beam sizes. For example, a recent study from Pelliccioli *et al.* (21) has shown that a perfect collimator assumption such as implemented in the hybrid dose calculation might introduce an underestimation of the calculated valley doses up to 30 %.

Animal behavior and quality of life (QoL)

Before the radiotherapy, 7 complete and 1 partial seizure occurred. After irradiation, the overnight monitoring did not indicate any adverse events. The day after treatment, the dog experienced 6 partial seizures. After 2 days of treatment with levetiracetam ($60\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$), the seizures completely stopped. Neither alopecia nor radiodermatitis were noticed. The monthly examinations by the veterinarians revealed no neurological changes during the first 3 months post irradiation. Typical side effects of the corticoid treatment (polyphagia, polyuria-polydipsia) occurred but

they were reduced after tapering of the prednisolone intake from 30 days after irradiation onwards. The owner survey showed that the QoL score of the animal increased from “fair (4/10)” before irradiation, to “medium (5.5/10)” 15 days after irradiation, and continued raising from “very good (7.5/10)” to “excellent (8.5/10)” from 30 days after treatment, and thereafter. The follow-up is currently ongoing.

Tumor response and imaging results

MRI performed at 1 and 3 months post irradiation did not reveal signal modification in normal tissues. MRT-induced tumor regression was already noticeable 1 month after exposure. Only the necrotic part of the tumor (fluid-like hypersignal) remained detectable, surrounded by a mild inflammation. At 3 months post irradiation, the inflammation pattern was reduced, the mass effect was minimized with normalization of the left lateral ventricle and the tumor volume reduction was 87.4% (Figure 4). Brain necropsy will be conducted after death to evaluate microscopic normal tissue and tumor responses.

Discussion and conclusions

As demonstrated in numerous preclinical studies (4, 6–8), spatially fractionated irradiation has significantly challenged the understanding of how normal and tumoral tissues respond to radiation therapy. Spatial fractionation of the incident x-ray into microbeams may allow to deliver significantly higher radiation doses (hGy in the microbeam paths) to the target when compared to conventional radiotherapy, without increasing normal tissue toxicity. In the past decades, the implementation of clinical standards on synchrotron x-ray treatment stations has progressed, in terms of medical physics and radiation delivery safety, and now allow a safe clinical transfer of the technique. A first large animal MRT study in healthy minipigs has shown that the radio-ablation of the right caudate nucleus could be safely and effectively performed (12). This first veterinary patient case-report on a dog with a spontaneous brain tumor confirms the radiotolerance of normal tissues during the (sub)acute phase after microbeam exposures. Indeed, despite high entrance doses, (peak doses ~ 60Gy), no edema nor Gd-DOTA diffusion were detected in normal tissues. Furthermore, the results demonstrate that deep seated (5-10cm depth) lesions can be treated with synchrotron-generated microbeams. To complete this translational study, a dose escalation is foreseen (2.8Gy, 5Gy and 7Gy minimum valley dose, with a ballistic optimization using up to 10 non-coplanar beams).

To our knowledge, this neuro-oncologic veterinary trial is the first 3D conformal synchrotron x-ray MRT treatment of spontaneous intracranial tumor in a large animal ever performed and realized under clinical conditions. It shows the feasibility and the short term innocuity of the technique. In this paper, we reported the first microbeam treatment of a deep seated spontaneous glioma in a dog patient, following a clinical workflow. This is the methodology and case report of a recently started animal patient

trial at the XXXX. The trial is scheduled to last until mid 2025 and will involve 27 dogs in a dose escalation scheme. This vet trial will generate essential toxicity and tumor response data which will pave the way of the clinical transfer of MRT in a synchrotron x-ray source in the near future.

Journal Pre-proof

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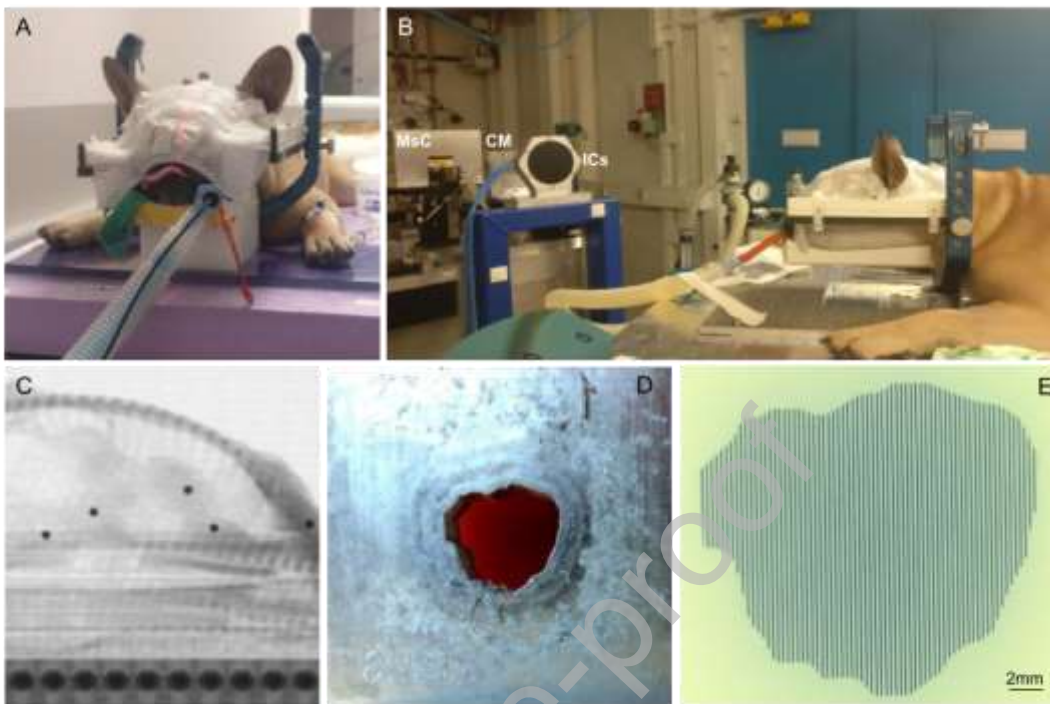
Figure and table captions

Figure 1: (A) Dosimetry scan in the veterinary clinic. The dog's head is maintained in a non-invasive stereotactic radiotherapy frame. Metallic beads are positioned on the mask for the MRT treatment positioning. (B) Irradiation set-up, multislit collimator (MsC), Cerrobend mask (CM, detailed on D), real time 2D ionization chambers (ICs) and the patient in treatment position for the first beam. (C) A Lipowitz alloy mask for conforming the microbeam array to the PTV. (D) One of the two synchrotron beam radiographs, acquired for isocenter positioning using the metallic markers that can be seen on the image. (E) Gafchromic film showing the conformal microbeam array treatment beam.

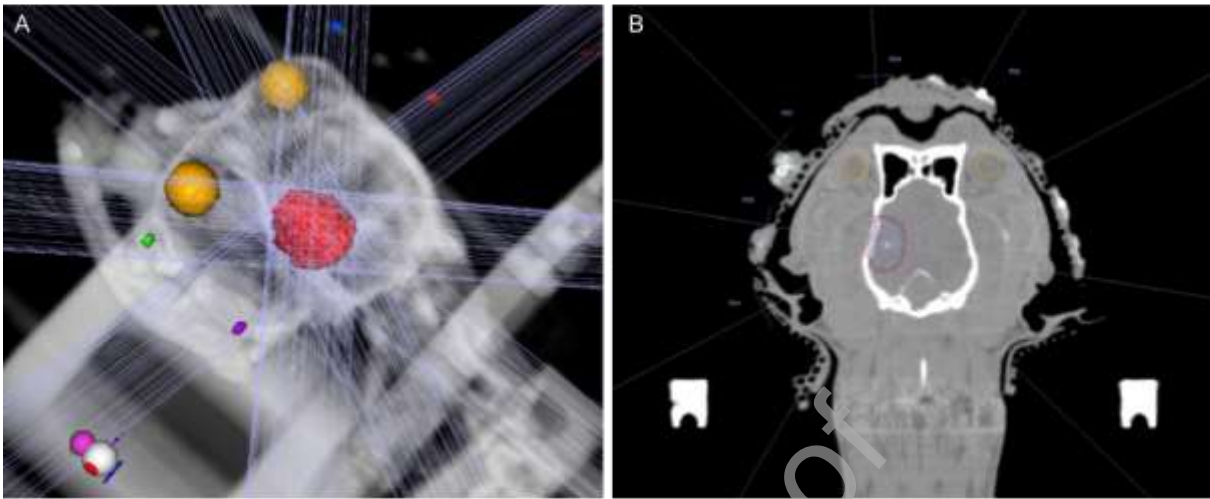


Figure 2: Treatment plan as seen on a 3D view (A) and on a coronal slice (B), including PTV, CTV, eyes and metallic markers, together with the 5 MRT conformal beams.

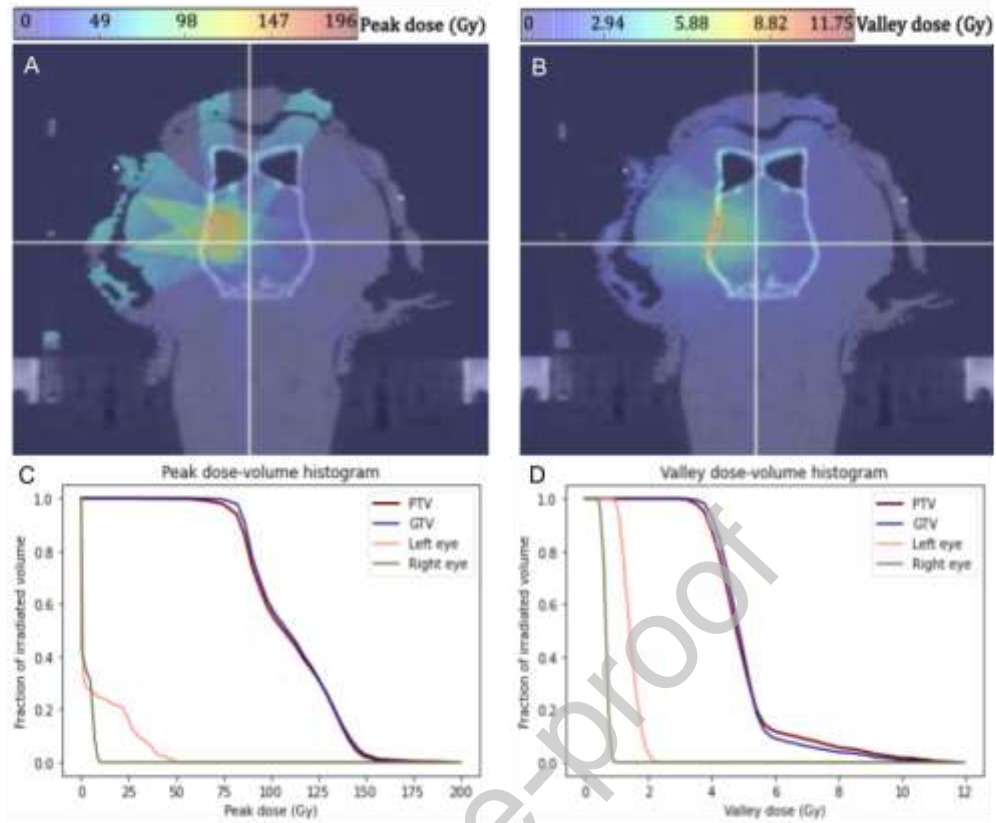


Figure 3 : (A) Peak dose map in the coronal slice at the isocenter level. (B) Valley dose map in the coronal slice at the isocenter. (C) Cumulated peak dose volume histogram and (D) Cumulated valley dose volume histogram.

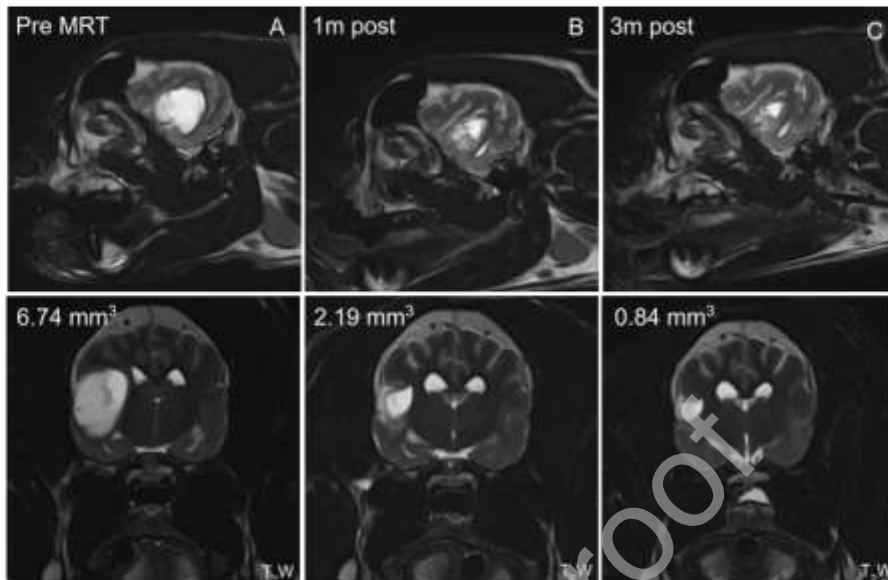







Figure 4 : MRI follow-up of the first MRT patient. Sagittal and axial T₂W images acquired before (A), one (B) and three (C) months after MRT treatment. Tumor volumes are reported in cm³.

Table 1: Planned doses for each treatment field. Experimental dosimetry: EBT3 film dosimetry in a solid water cube and relative errors with respect to hybrid dose calculations in the same conditions. N.A. : for low depth, the experimental peak dose maps are not available because of the sensibility range of the EBT3 films.

Fields	Depth (mm)	Calculated dose (Gy)	Measured dose (Gy)	Relative difference (%)
Field 1 	50	Peak: 33.024	N.A	N.A
		Valley: 1.53	Valley: 1.92 ± 0.14	Valley: 24.49 ± 9
Field 2 	60	Peak: 28.5703	N.A	N.A
		Valley: 1.33	Valley: 1.61 ± 0.11	Valley: 21.05 ± 8
Field 3 	70	Peak: 24.7168	N.A	N.A
		Valley: 1.25	Valley: 1.42 ± 0.08	Valley: 13.52 ± 6
Field 4 	90	Peak: 17.46	Peak: 15.78 ± 0.5	Peak: -9.62 ± 2
		Valley: 0.72	Valley: 0.88 ± 0.07	Valley: 22.22 ± 10
Field 5 	100	Peak: 15.51	Peak: 14.31 ± 0.5	Peak: -7.73 ± 3
		Valley: 0.72	Valley: 0.89 ± 0.08	Valley: 23.6 ± 11