CHUV | CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

Master's Thesis

Use of new biochemical plasmatic markers, plasma free and total metanephrines, for the diagnosis and follow-up of neuroblastoma in children with clinical correlation

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Abstract

Background:

Neuroblastoma is a paediatric tumour derived from the neural crest. Biochemical diagnosis and follow up rely on quantitation of urinary catecholamines (dopamine and noradrenaline) and their metabolites vanillylmandelic acid (VMA) and homovanillic acid (HVA) (gold-standard). When combined, these analyses have a sensitivity of 95%. However, they are clearly limited by inaccuracy of urine collection in young children and normalisation of catecholamine concentrations by creatininuria. Recent development in biochemical diagnosis of pheochromocytoma, another neural crest tumour found in adults, shows that plasmatic measurement of methoxylated catecholamines called metanephrines are more sensitive and specific than other biomarkers. Moreover, a study to determine the reference intervals for metanephrines in a pediatric population has recently been completed. The aim of this work is to describe the role of metanephrines monitoring in the follow up of neuroblastoma.

Method:

This retrospective study included patients with neuroblastoma in whom the following parameters were determined: plasma free and total metanephrines, plasma catecholamines, 24h urinary catecholamines and metanephrines in absolute value and corrected by creatinine, VMA and HVA at the diagnosis and during treatment at the University Hospital of Lausanne (Switzerland). Eleven patients aged between the first day of life and 7 years old were followed between 2005 and 2012. Clinical outcome and biochemical concentrations of the analytes were correlated.

Results:

At diagnosis, plasma free and total normetanephrines and methoxytyramine have a sensitivity of 100% compared to 85% for the actual gold standard. Metanephrine remain below the upper reference limit as expected since these tumours do not produce adrenaline.
The relationship between biochemical markers and clinical outcome is illustrated graphically. Plasma or urinary normetanephrine and methoxytyramine correlate better with the history of the patient than VMA and HVA, as evaluated by ordinal logistic regression. Concentrations of analytes in urine show a better correlation with clinical events when the results are corrected by creatininuria.

Conclusion:

Normetanephrine and methoxytyramine reflect disease history in neuroblastoma patients and could play a significant role in the follow up of this type of tumour. Formal studies in a sufficient number of patients are needed to confirm this preliminary observation.

Résumé

Introduction:

Le neuroblastome est une tumeur de l'enfant dérivant de la crête neurale. Le diagnostic biochimique et le suivi sont basés sur les dosages des catécholamines urinaires (dopamine et noradrénaline) et de leurs métabolites acides vanillylmandélique (VMA) et l'acide homovanillique (HVA) (analyses diagnostiques de référence). Ensemble, ils ont une sensibilité de 95%, mais l'inconvénient de cette technique est une collection incertaine des urines de 24 h ou bien d'un spot urinaire pour lequel les concentrations doivent être normalisées par la créatinine. De récents développements dans le domaine du diagnostic biochimique du phéochromocytome, une autre tumeur neuroendocrinienne observée principalement chez les adultes, montrent que le dosage plasmatique des catécholamines méthoxylées appelées métanéphrines est plus sensible et spécifique que les autres biomarqueurs. Une étude visant à déterminer les limites de référence pour ces paramètres dans une population pédiatrique a déjà été effectuée. Le but de ce travail est de décrire le rôle de la mesure des métanéphrines dans le suivi des neuroblastomes.

Méthode:

Cette étude rétrospective inclue des patients avec un neuroblastome chez lesquels les métanéphrines plasmatiques libres et totales, les catécholamines plasmatiques, les catécholamines et métanéphrines urinaires de 24h et normalisées par la créatinine ont été mesurées pour le diagnostic et le suivi clinique à l'hôpital universitaire de Lausanne (Suisse). Onze patients âgés du premier jour de vie à 7 ans ont été suivis entre 2005 et 2012.

Résultats:

Au moment du diagnostic, la normétanéphrine et la métoxytyramine plasmatique libre et totale ont une sensibilité de 100% contre 85% pour les dosages urinaires (méthode diagnostique de référence). Les concentrations d'analytes dérivant de l'adrénaline reste en-dessous la limite supérieure de la norme étant donné que ces tumeurs ne produisent pas d'adrénaline mais de la noradrénaline.
La relation entre les concentrations des marqueurs biochimiques et l'évolution clinique de ces patients a été étudiée graphiquement. La normétanéphrine et la méthoxytyramine plasmatique et urinaire décrivent mieux l'histoire du patient que l'acide vanillylmandélique et l'acide homovanillique, selon une analyse par régression logistique ordinale. La normétanéphrine et la methoxytyramine urinaires ont une meilleure performance quand elles sont corrigées par la créatininurie.

Conclusion:

La normétanéphrine et méthoxytyramine reflètent l'histoire clinique des patients atteints d'un neuroblastome et pourraient assurément jouer un rôle dans le suivi clinique de ces tumeurs. Une étude de plus grande envergure sur un plus grand nombre de patients devrait être réalisée afin de confirmer ces observations préliminaires.

Introduction

Neuroblastoma is the most common extra-cranial solid tumor in children and originates from the sympathetic nervous system. It shows a wide variety of biological activity and has a correspondingly diverse range of presenting features and prognosis. Patients below 18 months of age usually have a better outcome than older ones and require less intensive treatment, and indeed some children may not require any treatment at all, since the tumour can spontaneously regress. In contrast, older children often present with more advanced and biologically aggressive forms of the disease and the outcome is often poor despite very intensive treatment[1]. The primary sites are in the retroperitoneum (approximately 65% of the cases), posterior mediastinum (approximately 20%), pelvis (< 5%), and neck (<5%); sometimes, no primary site is identified. In 50% to 60% of the patients, the metastases are found in the cortical bone, bone marrow, lymph nodes, and/or liver, but they rarely spread to lung parenchyma or the CNS[2].

As for the clinical presentation, the symptoms are not specific (decline in general health, loss of appetite, fever, loss of weight, abdominal pain or, one out of two times, simply the detection of a mass at the first exam)[3]. The diagnosis is made by histological analysis of biopsy and elevated urinary catecholamines (noradrenaline and dopamine) and their acid metabolites: vanillylmandelic acid (VMA) and homovanillic acid (HVA). According to Candito[4], these measurements are considered to be the gold standard for diagnosis and follow-up. Metabolism of the catecholamines and their metabolites is depicted in figure 1.

According to Hartmann's study, 88% of the neuroblastoma are secreting one of the 3 urinary metabolites (HVA, VMA and dopamine) whereas the remaining tumors are metabolically inactive[5].

A more recent study established that combined urine catecholamines and their metabolites (VMA and HVA) have a very good sensitivity (95%)[6] . However, 24h-urine samples may not always be collected reliably, especially in neonates and paediatric patients. Therefore, a study from Pussard et al. in 2008 has determined reference values for age-dependent variations of creatinine and was thus able to take measurements on random untimed urine specimens by correcting them with urinary output of creatinine excretion[7].

In adults, a recent study evaluated plasma free and total metanephrines as diagnostic tool in pheochromocytoma[8] (another tumor derived from the neural crest) and showed that they were as sensitive and specific as urinary fractionated metanephrines. Thus, their value and role could be of interest in pediatric neuroblastoma.

A first study was carried out to establish reference values of free and total normetanephrines (NMN), free and total metanephrine (MN) and free and total methoxytyramine (MT) in healthy children and to compare with measurements in a few patients with neuroblastoma[9]. Based on these reference values, we reviewed 11 patients with neuroblastoma with the aim to compare laboratory values with clinical data at diagnosis and during treatment, and thus to describe the role of metanephrines monitoring in the follow up of neuroblastoma.

Materials and Methods

Study design and participants

This retrospective study included patients with neuroblastoma in whom plasma free and total metanephrines, plasma catecholamines, 24h urinary catecholamines and metanephrines and urinary catecholamines and metanephrines correlated with creatinine as well as VMA and HVA were measured for the diagnosis and during monitoring of the disease at the University Hospital of Lausanne (Switzerland). Eleven patients aged between the first day of life and 7 years were followed between 2005 and 2012.
All measurements were performed at the Laboratory of Clinical Pharmacology and Toxicology in Lausanne.

Clinical data are summarized in Table 1.

Laboratory analyses

Laboratory analyses are following the same methodology previously published[10].
Blood sampling. *All blood samples were collected by puncture with the patient kept seated for at least 20 min before sampling. Patients were instructed to fast and to abstain from chocolate beverages or bananas or acetaminophen overnight. All samples were collected onto ice and centrifuged within 30 min after puncture. Plasma was kept at −80 °C until analysis. Twenty-four-hour urinary metanephrines and catecholamines were collected into opaque bottles containing hydrochloric acid to ensure analyte stability.*
Analyses.*The plasma samples were analyzed by HPLC with coulometric detection for total metanephrines after acid hydrolysis [11] and plasma free metanephrines were quantified by tandem mass spectrometry[12]. Metanephrines in urine were analyzed by HPLC as total hydrolysis-fractionated normetanephrine and metanephrine [13]. Plasma catecholamines (norepinephrine and epinephrine) were analyzed using HPLC with amperometric detection [14] Catecholamines, VMA and HVA were determined by HPLC with amperometric detection (reference III)*."Reference values for plasma analyses were determined in a paediatric patient population in our institution after receiving the agreement from the ethical committee of the CHUV (figure 3). Reference values for urine parameters are shown in figure 4-5 (8).

Graphics and statistical analysis

The graphical analysis of the biomarker values are represented as the percentage over the upper reference limit normalized to the age of the patient. The established limits of reference values such as used by the laboratory of the CHUV in Lausanne are shown in table 2-4.
The various markers at diagnosis are expressed as absolute value and as ratio over their upper reference limit. Specificity of the biomarkers during the disease monitoring could not be determined as every patient had the disease. We focused on the following markers: the actual gold standard used at the CHUV (U NE, U DA, U VMA, U HVA) and the O-methylated lines (MN, NMN and MT in the plasma free (PF), total (PT) and urine (U) lines).
For monitoring of the disease, the laboratory values are expressed as ratio over their

upper reference limit and are compared according to RECIST score (complete remission (CR) partial remission (PR), progressive disease (PD)) and response of the treatment (chemotherapy and surgery).

Statistical analysis for the follow up allowed comparing the performances of the different biomarkers available with regard to clinical evolution and treatment response. Clinical outcomes were coded as RECIST scores (1-4), considered as ordinal variable, while the biomarkers were expressed first as log-transformed absolute laboratory values and, in a second step, as log-transformed ratios of percentage of the upper reference normalized for age of the patient. A model of ordinal logistic regression, which still included the repetition of tests within each patient, was finally applied to relate each biomarker to the outcome along follow up time.
We used for this intent an "ordered logit" model. We began by exploring whether time itself had an effect. The longitudinal formulation of this model takes into consideration intrapatient correlation. We omitted the diagnosis period (RECIST=0, which trivially occurs in early follow up).
We explored all biomarkers with the same model to compare their respective performance for outcome prediction.
Statistical analyses were performed using STATA (v10, Statacorp, College Station, TX, USA).

Results

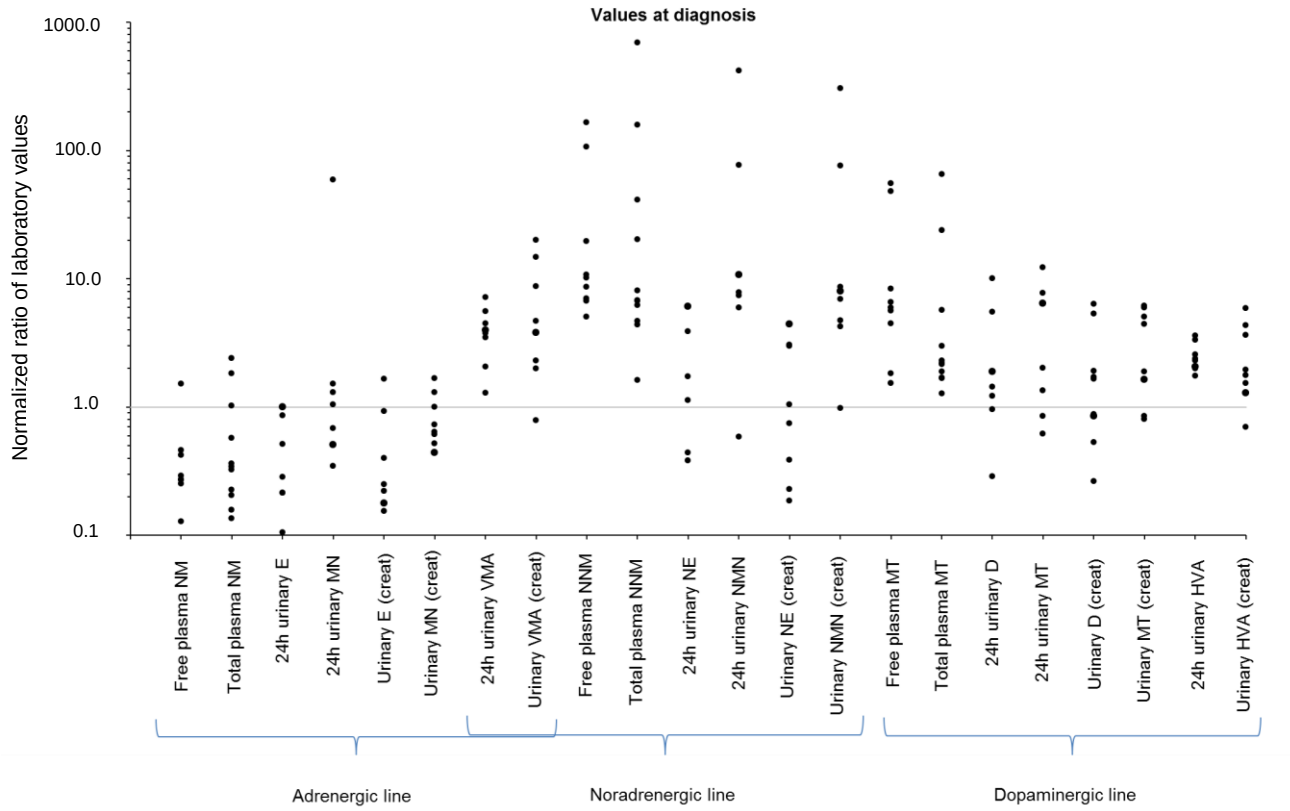
Comparison of the biomarkers at diagnosis

Variation of biomarkers in the population

Eleven patients (patient 1-11) diagnosed with neuroblastoma were analysed. Their age was (mean ± SD) 1.13 ± 1.60 years. Twenty-two different biomarkers were measured for diagnosis, Plasma free MN, NMN and MT were measured in patient 3 to 11. Total plasma MN, NMN and MT were measured in all patients. 24h urinary E, NE, DA, HVA and VMA were measured in patient 1, 3, 4, 5, 6, 7, 10, 11. 24h urinary NE were measured in patient 1, 3, 4, 5, 7, 11. 24H urinary MN, NMN, MT were measured in patient 1, 3, 4, 6, 7, 10, 11. Urinary E, NE, DA normalized to the creatinine were measured in patient 1, 2, 3, 4, 6, 7, 8, 9, 11. Urinary NE normalized to the creatinine was measured in patient 1, 2, 3, 4, 7, 8, 9, 11. Urinary MN, NMN, MT normalized to the creatinine were measured in patient 1, 2, 3, 4, 6, 7, 8, 11. Urinary HVA and VMA normalized to the creatinine were measured in patient 1, 2, 3, 6, 7, 8, 9, 11.

The most abnormal values reflecting a diagnostic potential are presented in Figure 2. It appeared that the parameters that are the most above their URL are fNMN, tNMN, fMT and tMT. VMA and HVA were also above their respective URL but with less amplitude. U NE, U DA and U NMN were disappointing since 2 patients were detected as negatives.

Figure 2: Catecholamines and derivatives express as fraction of the upper reference limit



The statistics analysis of the adrenergic line's biomarkers is shown in table 5-6. According to the literature, neuroblastoma does not produce the adrenergic line excepted for the VMA who derives from the noradrenergic line, too. Similarly, we found the same with our patients since the median of the adrenergic line ranged between 22% for U E and 105% for 24h U MN, but there is just one value very high (patient 6: 5950. %) with the rest of the medians not higher than 68% (U MN). These results were arbitrary used as the baseline of the physiological catecholamine production. For every biomarker there is always at least one patient upper the URL. Strikingly, the fNMN or tNMN are more than 2000% of the upper reference limit.

Noradrenergic line data are shown in the table 7-8. fNMN (1st quartile 702%, median 1022%) and tNMN (1st quartile 546%, median 677%) have a better sensitivity (100%) than U NE(50%) and U VMA (87%). The U NE has a median of 89% of the URL.

Dopaminergic line data are shown in table 9-10. fMT (1st quartile 447%, median 596%) and tMT (1st quartile 179%, median 220%) have a better sensitivity (100%) than U D sensitivity (55%) and U HVA sensitivity (87%).

U NE (median 89%) and U DA (median 166%) provide a sensitivity of 50% and 55%. The combination of U VMA and U HVA gave a sensitivity of 100% but two patients (2 and 8) have just one of each marker below the reference limit when adjusted to the creatininuria.

The combination of the noradrenergic and the dopaminergic line provided the best sensitivity indicating that neuroblastoma preferentially produced these metabolites. In this study, fNMN and fMT have both 100% of sensitivity, the same for tNMN and tMT. But the median of plasma free line concentrations is higher than the median of plasma total line.

24h U VMA (1st quartile 314%, median 387%) and HVA (1st quartile 204%, median 235%)

have a combined sensitivity of 100%.

Normalized ratio of adrenergic line	Adrenergic Line							
	F P MN	T P MN	24h U E	24h U MN	U E	U MN	24h U VMA	U VMA
Mean	0.4	0.7	0.4	9.3	0.4	0.9	4.0	7.2
Max	1.5	2.4	1.0	59.5	1.7	1.7	7.2	20.1
Min	0.1	0.1	0.1	0.3	0.0	0.4	1.3	0.8
Standard deviation	0.4	0.8	0.4	22.2	0.5	0.4	1.9	6.9
Quartile(25 centile)	0.3	0.2	0.2	0.6	0.2	0.6	3.1	2.2
Median	0.3	0.3	0.3	1.1	0.2	0.7	3.9	4.3
Quartile(75 centile)	0.4	0.8	0.6	1.4	0.4	1.1	4.8	10.3
Sensibility	0.1	0.3	-	0.6	0.1	0.4	1.0	0.9

Table 5: statistics with the adrenergic line at diagnosis, the values are expressed as a ratio over their upper reference limit.

	Adrenergic Line							
	F P MN	T P MN	24h U E	24h U MN	U E	U MN	24h U VMA	U VMA
mean	0.16	5.27	7.00	1'445.86	18.89	324.13	58.25	96.25
max	0.59	20.86	17.00	8'926.00	75.00	836.00	101.00	281.00
min	0.07	0.92	1.00	52.00	1.00	96.00	18.00	11.00
standard deviation	0.17	6.68	6.28	3'299.52	24.38	272.24	25.69	100.07
median	0.11	2.28	3.50	220.00	8.00	217.00	61.50	52.00

Table 6: statistics are done with the direct laboratory values

Normalized ratio of noradrenergic line	Noradrenergic Line							
	F P NMN	T P NMN	24h U NE	24h U NMN	U NE	U NMN	24h U VMA	U VMA
mean	37.8	86.7	2.3	75.8	1.6	52.0	4.0	7.2
max	165.5	694.6	6.1	421.2	4.5	306.5	7.2	20.1
min	5.1	1.6	0.4	0.6	0.2	1.0	1.3	0.8
standard deviation	57.7	206.7	2.3	154.6	1.6	105.8	1.9	6.9
quartile(25 centile)	7.0	5.5	0.6	6.7	0.3	4.6	3.1	2.2
median	10.2	6.8	1.4	7.9	0.9	7.5	3.9	4.3
quartile(75 centile)	19.7	30.8	3.3	43.9	3.0	25.5	4.8	10.3
sensibility	1.0	1.0	0.7	0.9	0.5	0.9	1.0	0.9

Table 7: statistics with the noradrenergic line at diagnosis, the values are expressed as a ratio over their upper reference limit.

	Noradrenergic Line							
	F P NMN	T P NMN	24h U NE	24h U NMN	U NE	U NMN	24h U VMA	U VMA
mean	39.02	930.87	200.50	41'689.14	309.13	135'648.50	58.25	96.25
max	175.41	7'432.00	607.00	227'471.00	857.00	837'429.00	101.00	281.00
min	3.16	20.60	23.00	317.00	31.00	1'144.00	18.00	11.00
standard deviation	61.82	2'210.84	215.26	83'109.70	350.19	292'227.51	25.69	100.07
median	9.34	85.55	148.00	5'901.00	136.50	5'125.50	61.50	52.00

Table 8: statistics are done with the direct laboratory values

Normalized ratio of dopaminergic line	Dopaminergic Line							
	F P MT	T P MT	24h U DP	24h U MT	U DP	U MT	24h U HVA	U HVA
mean	15.4	10.1	2.7	4.5	2.2	3.4	2.5	2.6
max	55.7	65.2	10.0	12.3	6.3	6.2	3.6	5.9
min	1.5	1.3	0.1	0.6	0.3	0.8	1.8	0.7
standard deviation	20.9	19.4	3.4	4.5	2.2	2.3	0.7	1.8
quartile(25 centile)	4.5	1.8	0.8	1.1	0.9	1.4	2.0	1.5
median	6.0	2.2	1.3	2.0	1.7	3.2	2.4	1.9
quartile(75 centile)	8.4	4.4	2.8	7.1	1.9	5.3	2.8	3.8
sensibility	1.0	1.0	0.6	0.7	0.6	0.8	1.0	0.9

Table 9: statistics with the noradrenergic line at diagnosis, the values are expressed as a ratio over their upper reference limit

	Dopaminergic Line							
	F P MT	T P MT	24h U DP	24h U MT	U DP	U MT	24h U HVA	U HVA
mean	1.19	69.15	2'944.13	2'278.00	2'247.33	3'392.13	43.25	50.75
max	4.45	425.78	12'042.00	4'784.00	4'688.00	9'284.00	70.00	118.00
min	0.11	12.37	49.00	774.00	395.00	1'214.00	28.00	14.00
standard deviation	1.69	125.35	4'241.32	1'352.79	1'464.56	3'196.30	14.02	37.51
median	0.42	19.78	999.50	2'094.00	2'495.00	1'849.50	39.00	34.50

Table 10: statistics are done with the direct laboratory values

Comparison between the gold standard and metanephrines for the monitoring of the disease in patients

Clinical history and monitoring of the disease of patients are shown in the following graphs in figures 3-10. Patients are classified by final RECIST score: progressive disease (PD), partial remission (PR), complete remission (CR). For each patient, 4 graphs are done with different biomarkers:

- Gold standard: NE, DA, HVA, VMA, panel A
- Plasma total MN, NMN, MT, panel B
- Plasma free MN, NMN, MT, panel C
- Urinary MN, NMN, MT, panel D

We followed the variation of each biomarker in correlation with therapeutic interventions and RECIST score for each patient.

- During the progressive disease, we expect an elevation of the biomarker upper the URL.
- During the partial remission, we expect to detect a difference between the other periods.
- During the remission, we expect the biomarkers stay under the URL.

PD patients:

Patient 3:

The diagnosis was made at 40 months of life. A Chemotherapeutic protocol was immediately initiated. No decrease of the size of the tumour was noticed after receiving 2 different cures while after the surgical resection, he was in complete remission, but still received radiotherapy as adjuncted treatment. Less than one year later, he had a recurrence of metastasis, had a last cure of chemotherapy which had a short effect. Two and half years after the onset of the disease, he died.

The 4 graphs depicted on figure 3 showed that the markers also had the same tendency to fail after the first cure of chemotherapy. For the first recurrence, only the fNMN shows an increase over the URL. All analytes showed an increased concentration during the second recurrence but plasma free line has the most impressive rise being upper 10 times above the range (panel C),better than DA (highest value for the gold standard) which has a value 2.5 times above the range.

Patient 8:

The diagnosis was made the first day of life, two months later he received his first chemotherapy. But a half year after, the primary tumour and the metastases progressed.

He received 4 more chemotherapy cycles and had a surgery before he was in complete remission. Two years later, MIBG detected an infiltration of the bone marrow. We stopped the study at this time.
The 4 graphs depicted on figure 4 showed that during the first recurrence tNMN, HVA and VMA values were 10 times above the range (panel A and B). During the complete remission, all analytes have a tendency to fall; plasma total line stays borderline around the URL after the first recurrence (panel B). The second recurrence is only revealed with fMT (panel C), plasma total line has a tendency to elevate his values (panel B), but the gold standard stays below its URL (panel A).

PR patients:

Patient 5:

The diagnosis was done at 7 months of life, one month later, he began a chemotherapy. After that, he was in partial remission since the primary mass was still detected by the MRI.
All lines, depicted on figure 5 fall after the diagnosis and all stay borderline within the URL during the partial remission.

Patient 11:

The diagnosis was done at 4 years and 9 months of life. A first chemotherapy treatment followed by surgery resulted in partial remission. 6 months later, he received another chemotherapy cure. His last MIBG still showed few captation of the primary tumour and metastases (already present at diagnosis).
All lines, depicted on figure 6 gradually decreased after the first chemotherapy and stay below the range during the partial remission, except for fMT (panel A) and HVA (panel C) that were above their URL.

CR patients:

Patient 4:

The diagnosis was done at 10 months of life. The patient received one cycle of chemotherapy and after 3 months of disease was in complete remission and remains as it after 5 years (the longest follow up of this study).
All graphs depicted on figure 7 showed a quick fall of all biomarkers after the chemotherapy. During the complete remission, the gold standard (panel A) and the U line (panel D) stay under the range. Plasma free (panel C) and plasma total (panel B) lines stay borderline and occasionally cross the upper reference limit. All analytes overlap their concentrations with the adrenaline line known to not be involved in neuroblastoma biochemistry.

Patient 6:

The diagnosis was made at 1 month of life. He directly received chemotherapy and after 6 months, he was in complete remission.
The 4 graphs depicted on figure 8 showed that all analytes sharply decreased after chemotherapy and remain below their URL

Patient 7:

The diagnosis was made at 22 months of life. He directly received one chemotherapy cure and after 5 months had a laminectomy. 5 months later he had a complete resection of the primary mass.
The 4 graphs depicted on figure 9 showed that all analytes sharply decreased after chemotherapy and remain below their URL

Patient 10:

The diagnosis was made the first day of life. He directly had a surgery followed by 2 cures of chemotherapy and was in complete remission.
Unfortunately, we don’t have any urinary values at diagnosis (depicted on figure 10, panel

A and D). Of note, plasma free and total lines were 100-700 times above the range. The 4 graphs during the complete remission showed that all analytes sharply decreased after chemotherapy and remain below their URL

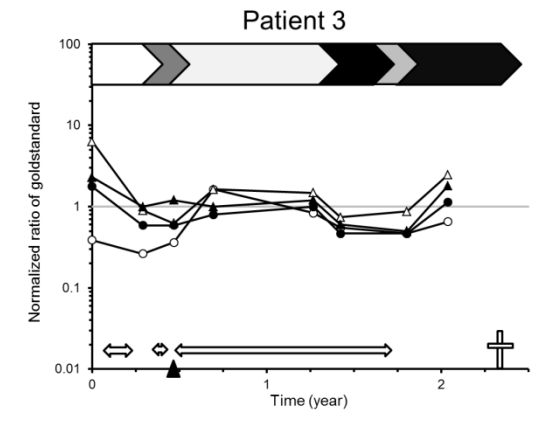
Results of the ordinal logistic regression

Time itself after diagnosis time seems to increase the probability of aggravation but to a statistically non-significant extent (p = 0.16). The results of the ordinal logistic regression analysis performed on each time series of biomarker values are shown in table 11, which gives the odds ratio associated with one log increase in the marker value along with the corresponding statistical significance p.value. According to this analysis, the best biomarker for the noradrenergic metabolic line is the urinary NMN (p=0,001) and the best biomarker for the dopaminergic line is the F MT (p=0,012).

Line	Adrenergic		Noradrenergic		Dopaminergic	
	val.abs.	ratio norm.	val.abs.	ratio norm.	val.abs.	ratio norm.
P F	.	.	1.59 p=0.07	1.62 p=0.07	1.64 p=0.08	**1.86** p=0.012
P T	.	.	.	.	2.13 p=0.06	**2.54** p=0.02
24h U Catecholamines	.	0.47 p=0.03	.	.	3.30 p=0.08	3.34 p=0.10
U Catecholamines	0.44 p=0.07	.	.	.	.	**6.43** p=0.02
24h U Metanephrine	.	.	**3.84** p=0.001	**2.83** p=0.004	**3.60** p=0.011	.
U Metanephrine	.	.	**2.16** p=0.008	**5.24** p=0.001	**3.21** p=0.005	**3.61** p=0.013
24h U acid	(NA)		**2.62** p=0.02	**2.23** p=0.04	**3.33** p=0.008	**2.78** p=0.03
U acid			1.80 p=0.08	**2.31** p=0.03	**2.34** p=0.05	**3.57** p=0.005

Table 11: Statistical results

Figure 3, A :



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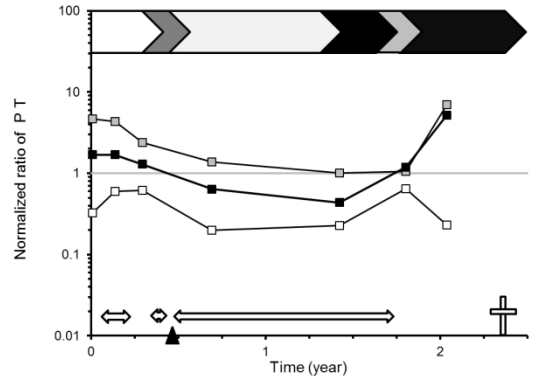

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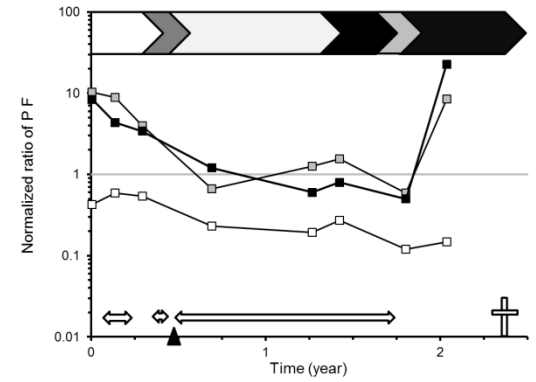
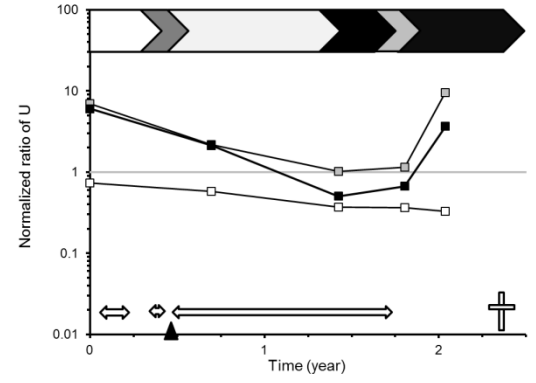


D :



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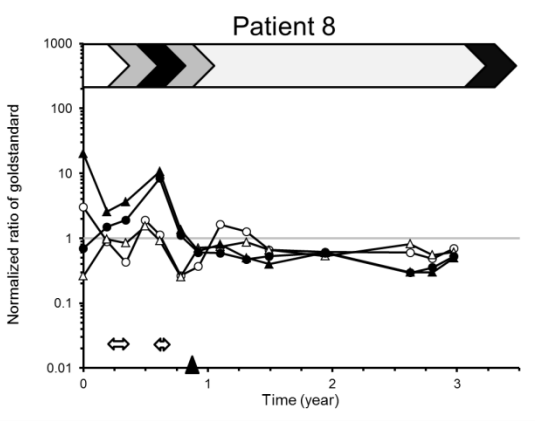

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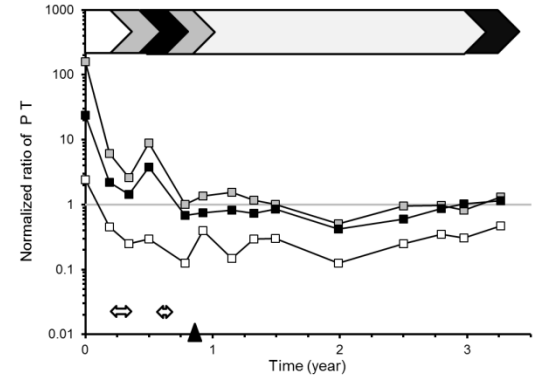

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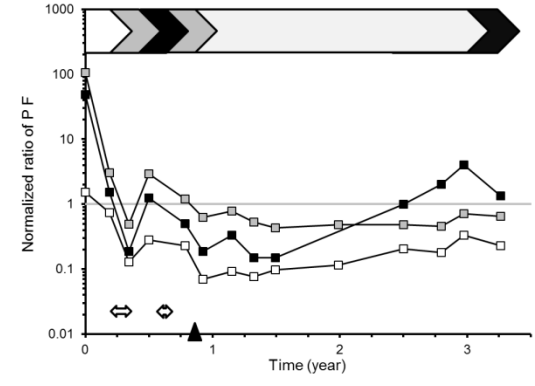

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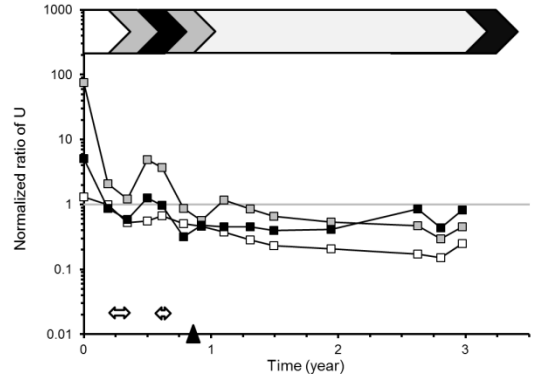

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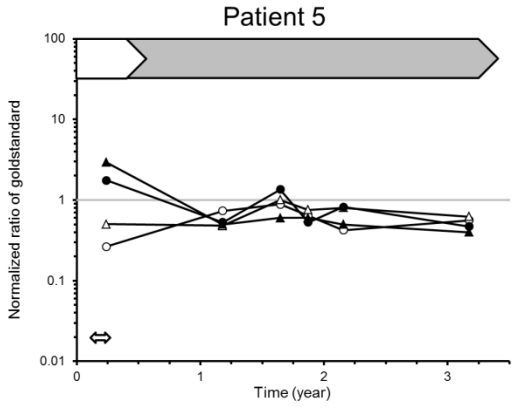

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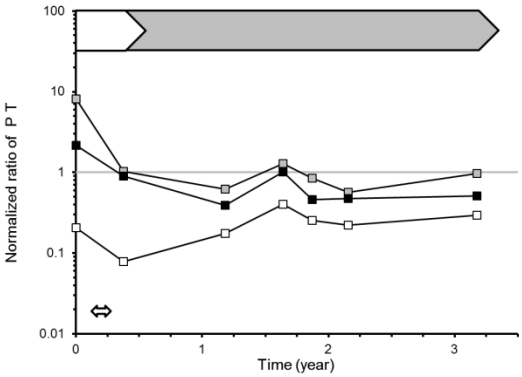

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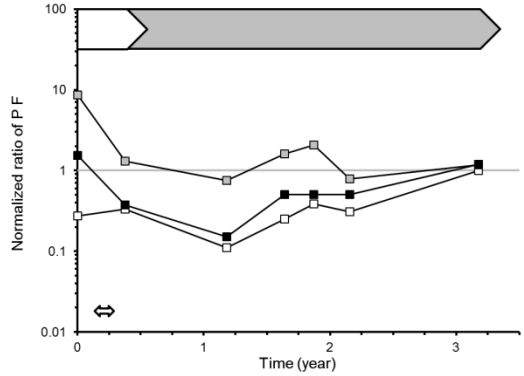

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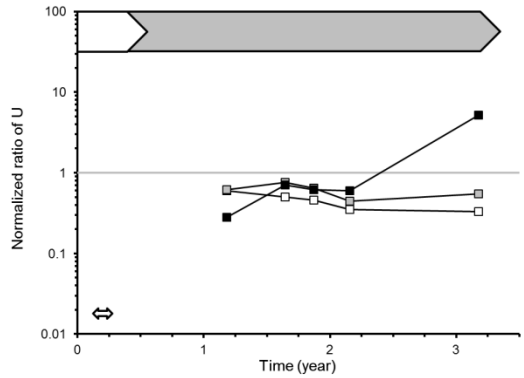

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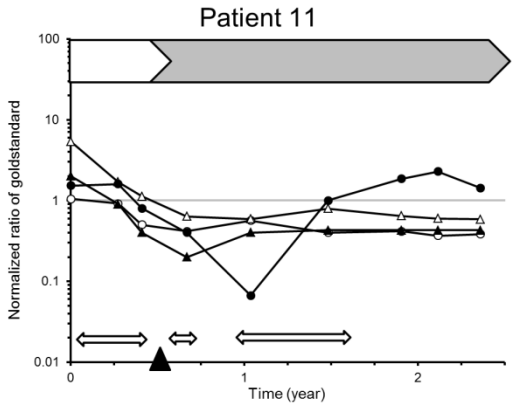

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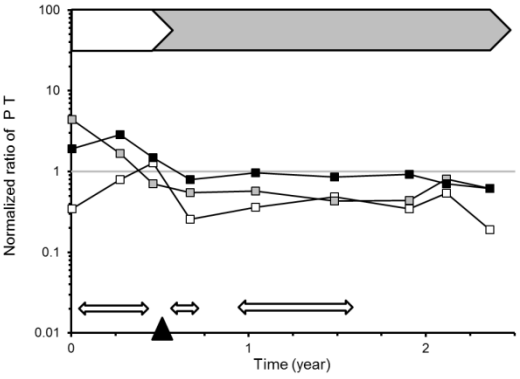

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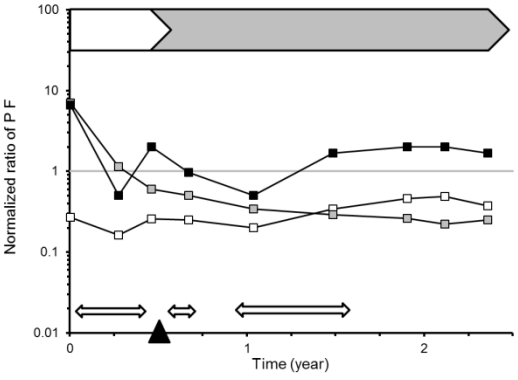

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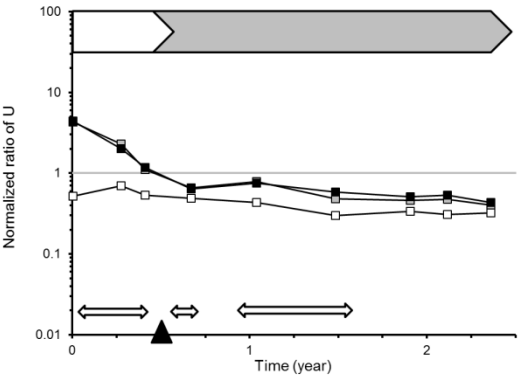

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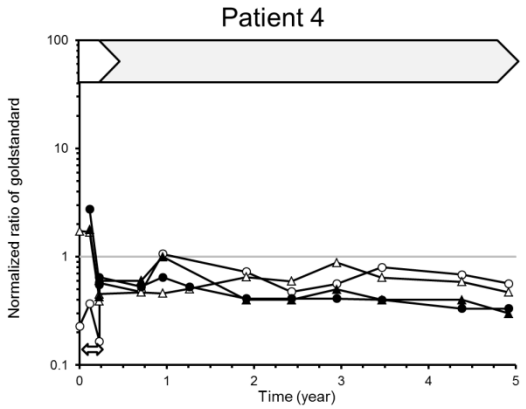

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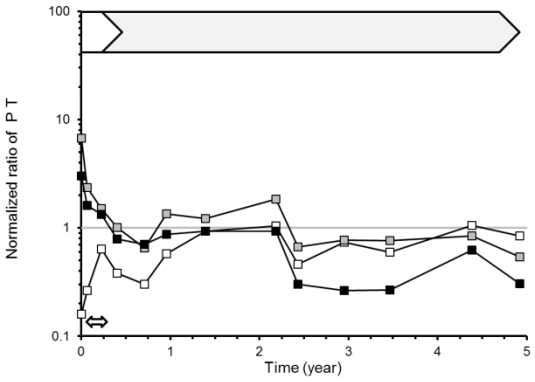

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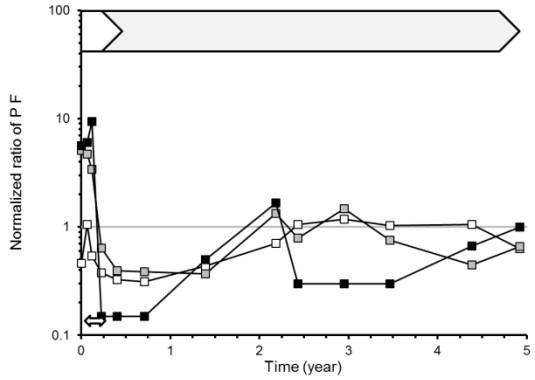

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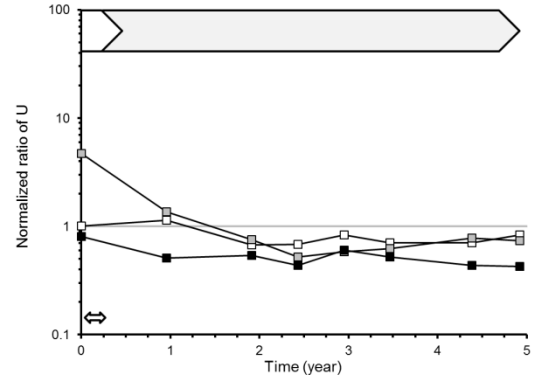

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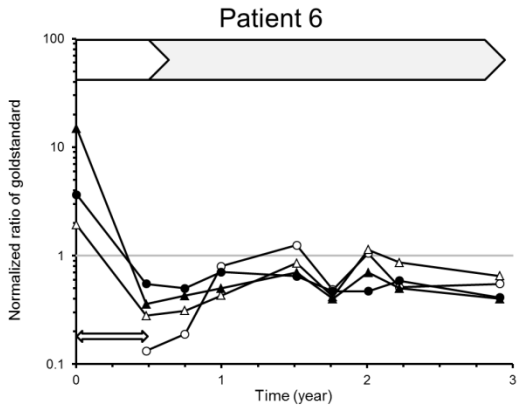

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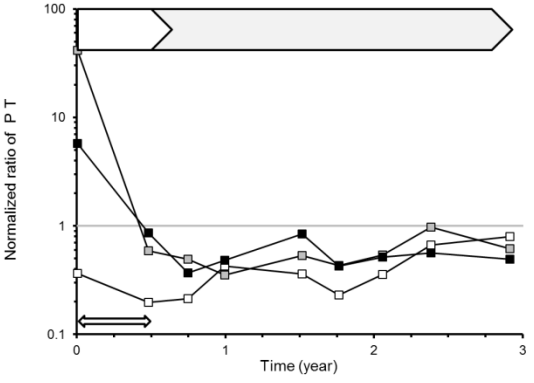

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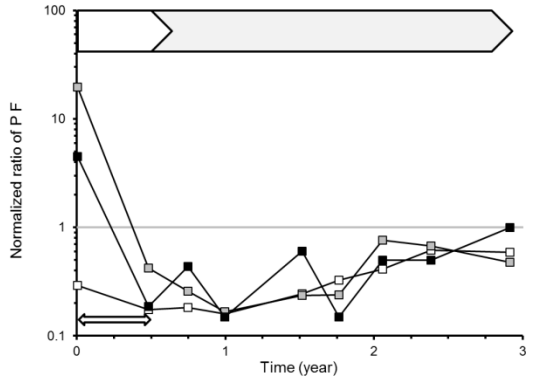

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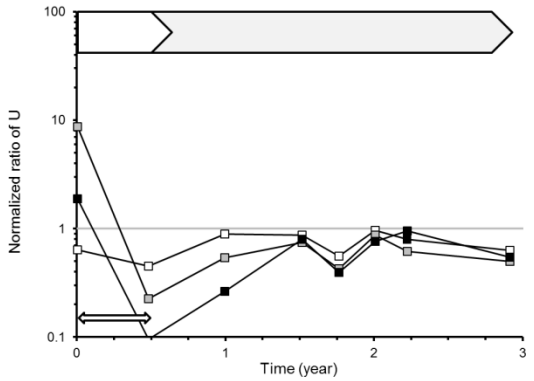

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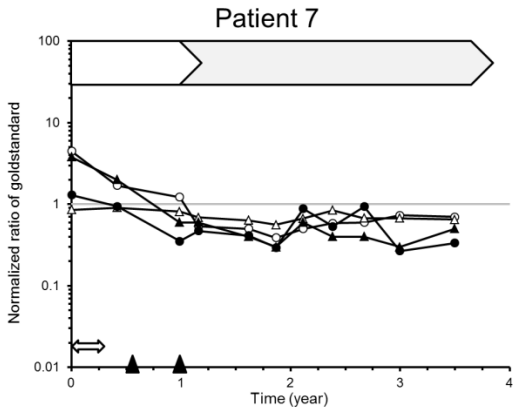

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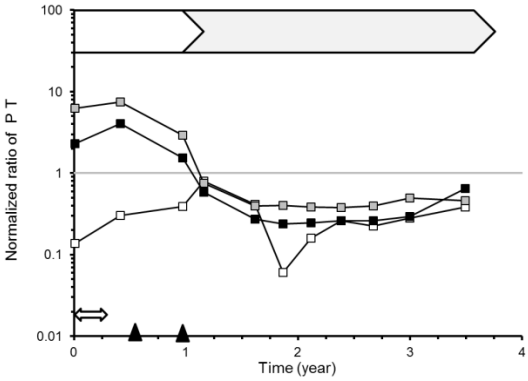

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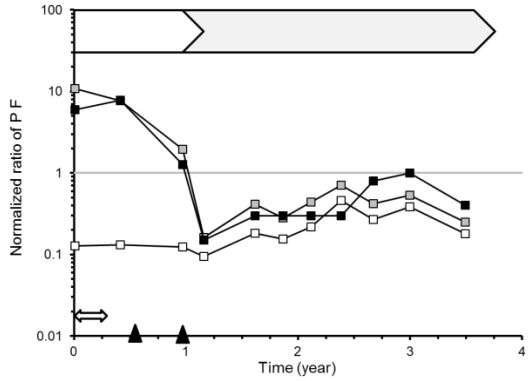

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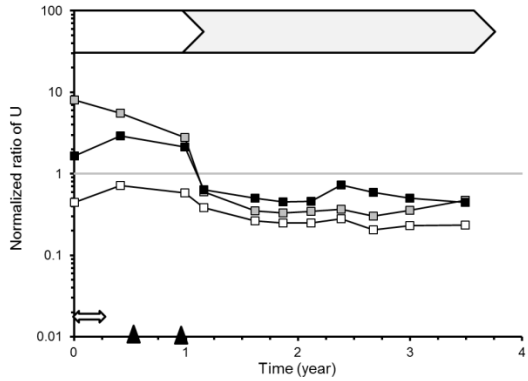

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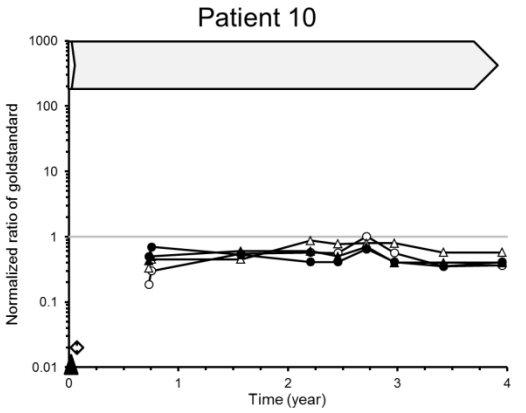

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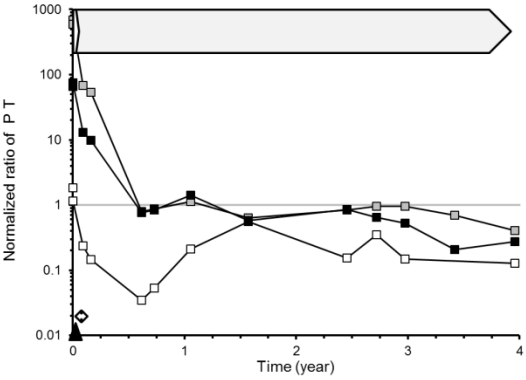

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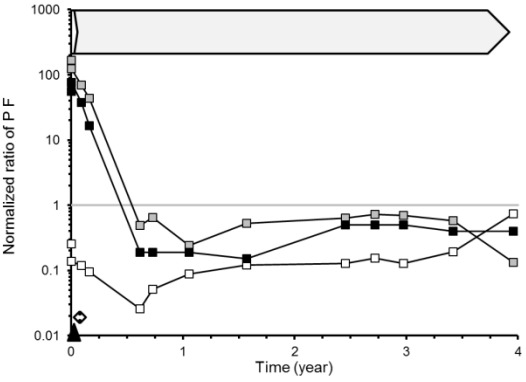

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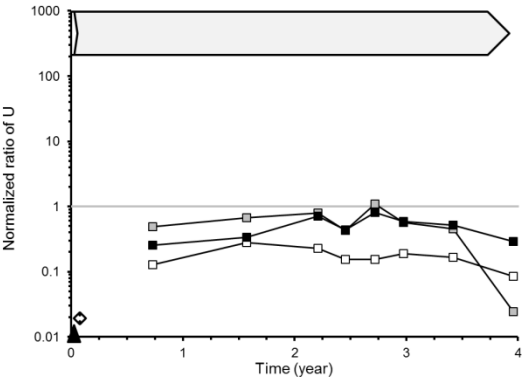

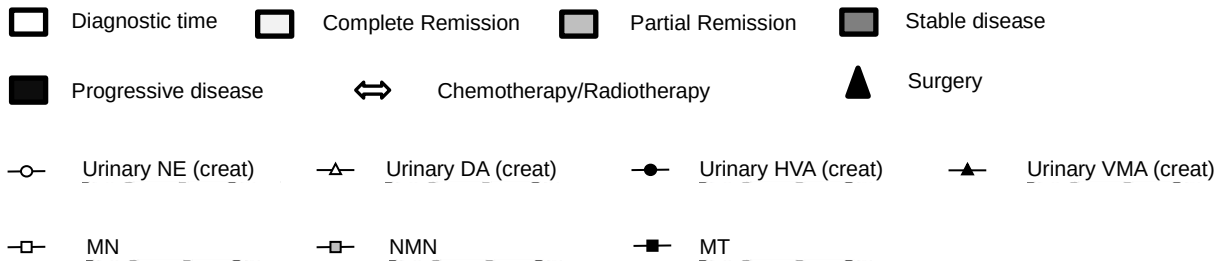

Figure 3-10: Monitoring of patient 3, 4, 5, 6, 7, 8, 10, 11 by U NE, U DA, U HVA, U VMA for the gold standard and MN, NMN and MT in P F, P T and U, expresses as fraction of the upper reference limit. RECIST score and treatment.

Discussion

This is the first study reporting longitudinal profiles of plasma free and total metanephrine concentrations in neuroblastoma paediatric patients in relation with clinical outcome. Since the size of the population investigated is small, we stay descriptive and have made few statistics that indicated some trends favouring plasma metanephrines monitoring to follow up the patients.

At diagnosis

According to the literature[15], HVA and VMA concentrations normalized by creatinine present a good sensitivity of 95% at the diagnosis. With our small group of patients, we find a sensitivity of 100% for this combination, but 18% of our patients just have a positive value with VMA or HVA. However, the urinary values are clearly limited by inaccuracy of urine collection in young children and normalisation of concentrations by creatininuria. To overcome this problem, plasma sampling appears more confident and indeed, at diagnosis, plasma free NMN and MT or plasma total NMN and MT are over the upper reference limit for each patient providing 100 % of sensitivity. Plasma free line has higher values at diagnosis than plasma total line in comparison to their respective upper reference limit.
The use of urinary catecholamines (NE and DA) for diagnosis is disappointing since we observed a sensitivity of 50% and 55%, respectively.
A prospective randomised study including more patients should confirm these preliminary observations.

Monitoring of the disease

Plasma free line seems to represent the best the clinical history. It shows every recurrence, metanephrines concentrations fall after a treatment and is the only line which detects a difference between a complete remission and a partial remission. Plasma total NMN + MT and urinary NMN + MT adjusted to the creatinine show the same trend but, during the complete remission, the urinary line seems to have less variation.
HVA and VMA of the gold standard are good biomarkers for the diagnosis, although less clearly than the plasma line. However, VMA and HVA have less sensitivity during the follow up of the patients most probably because they are a metabolic endpoint of catecholamine

produced by the tumors but also deriving from the diet. Nevertheless, all markers don't show every recurrence and we can't differentiate a complete of a partial remission based on biochemical measurements.

As predicted from the literature[5], we found that plasma free and total MN stay under the upper reference limit during all period investigated since neuroblastoma did not produce adrenaline.
We encountered some difficulties during this study. The first one was to validate the RECIST for each value. RECIST criteria are made for adult oncology and not children, this was described in McHugh's papers[16],[17]. The second one was to select criteria to analyse. There are a lot of different markers and there aren't enough studies about the monitoring to make any proposition of guide lines according to a systematic review of molecular and biological tumor markers in neuroblastoma[18]. The third one was to analyse the laboratory values expressed as a ratio over their URL. The URL of the F P and T P are not yet finalized and the paper still in preparation. The forth one was to combined two biomarkers because of the production of noradrenergic and dopaminergic catecholamine in neuroblastoma.

Conclusion:

Normetanephrine and methoxytyramine are clearly associated to disease history in neuroblastoma patients, with a good sensitivity and specificity at the time of diagnosis, and a significant association with clinical evolution during follow up.

Formal studies in a larger number of patients are needed to confirm this preliminary observation and to precisely assess the role of these biomarkers in the post-treatment monitoring of these patients.
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Figures and Tables

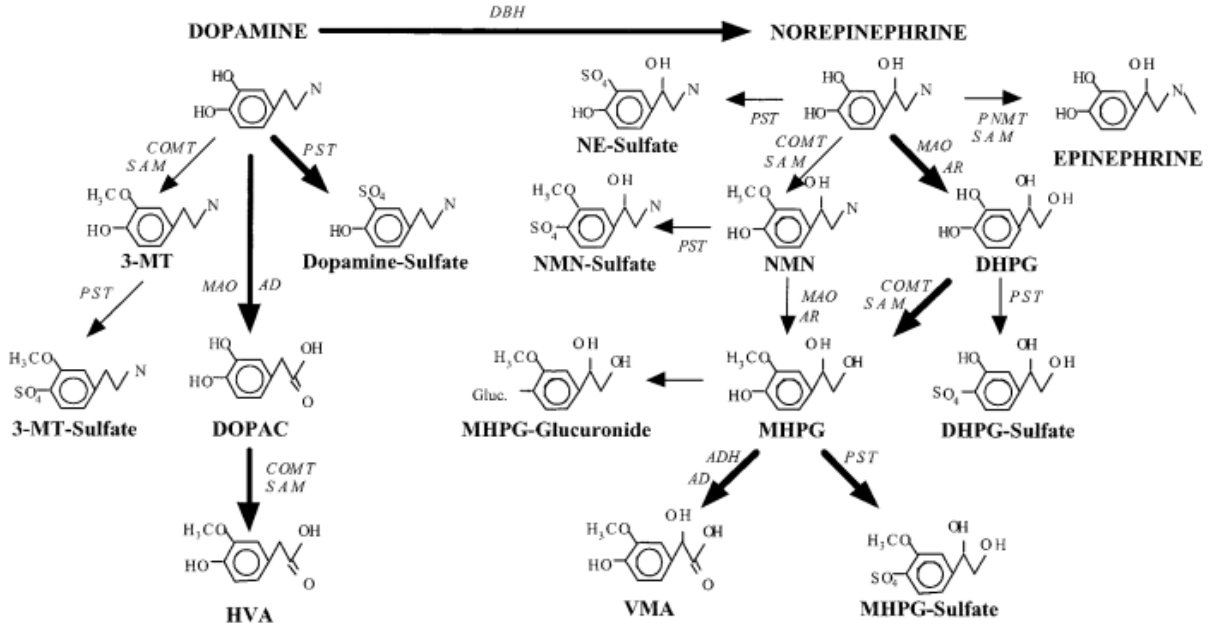


Figure 1: Metabolism of catecholamine, the minor metabolites, dihydroxymandelic acid (DHMA) and dihydroxyphenylethanol (DOPET), are not shown. AD, aldehyde dehydrogenase; AR, aldehyde reductase; NMN, normetanephrine; SAM, S-adenosyl methionine. [19]

[19] David S. Goldstein, Graeme Eisenhoher, and Irwin J. Kopin, *Sources and Significance of Plasma Levels of Catechols and Their Metabolites in Humans.* Received January 28, 2003; accepted March 18, 2003

Abbreviations list			
fMN	Plasma free metanephrine	tMN	Plasma total metanephrine
fNMN	Plasma free normetanephrine	tNMN	Plasma total normetanephrine
fMT	Plasma free methoxytyramine	tMT	Plasma total methoxytyramine
E	Epinephrine	DA	Dopamine
NE	Norepinephrine		
HVA	Homovanillic acid	VMA	Vanillylmandelic acid
24h U	24h urinary	U	Urinary adapted to creatinine
CR	Complete Response	PR	Partial Response
SD	Stable Disease	PD	Progressive Disease

Table 1:

pt nb	age at dg (m)	stage	histology	*MYCN*	other	treatment	outcome
1	0	4S	FH	-	-	no	CR
2	10	4	FH	-	1pdel	no	PD
3	40	4	-	-	-	CT, RT, S	PD
4	10	4	IH	-	-	CT	CR
5	7	4s	FH	-	-	CT	PR
6	1	3	-	-	-	CT	CR
7	22	3	FH	-	-	CT, S	CR
8	0	4s	-	-	-	CT, S	PD
9	0	4s	FH	-	-	S	CR
10	0	4s	-	-	-	CT, S	CR
11	57	3	UF	-	-	CT, S	PR

m: months
CT: chemotherapy
RT: radiotherapy
S: surgery
FH: favourable histology (according to Shimada)
IH: intermediate histology
UH: unfavourable histology

Table 2: Upper reference limit determined by CHUV's laboratory[1]

Plasma	Free (nmol/l)			Total (nmol/l)		
	MN	NMN	MT	MN	NMN	MT
0-1 an	<0.39	<1.06	<0.08	<8.68	<10.7	<6.53
1-2 ans	<0.61	<0.96	<0.1	<6.74	<8.94	<6.66
2-3 ans	<0.39	<0.46	<0.03	<4.44	<5.68	<4.84
3-5 ans	<0.26	<0.45	<0.05	<6.64	<9.86	<11.68
5-8 ans	<0.35	<0.76	<0.03	<5.14	<10.14	<4.86

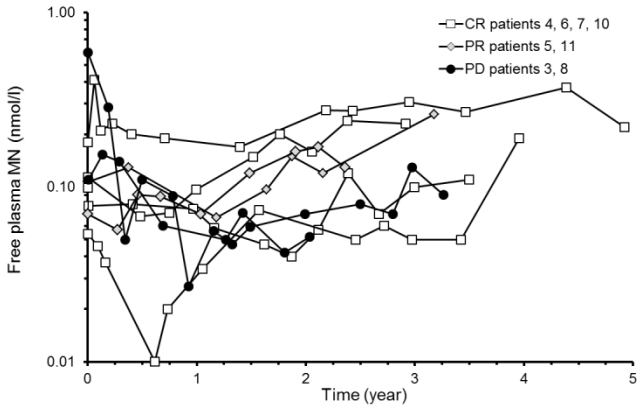
Table 3: Upper reference limit used by CHUV's laboratory for VMA and HVA

URINES		*VMA*	*HVA*
0 - 1 ans	umoles/24 h	< 14	< 16
	umoles/mmoles créat	< 14	< 20
1 - 2 ans	umoles/24 h	< 16	< 18
	umoles/mmoles créat	< 10	< 17
2 - 6 ans	umoles/24 h	< 16	< 21
	umoles/mmoles créat	< 10	< 15
6 - 16 ans	umoles/24 h	< 21	< 28
	umoles/mmoles créat	< 7	< 7
Adultes	umoles/24 h	< 25	< 34
	umoles/mmoles créat	< 3	< 4

Table 4 : Upper reference limit used by CHUV's laboratory for urinary catecholamined and O-methylated urinary line

URINES		NE	E	DA	NMN	MN	MT
0 - 3 mois	nmol/24h	< 60	< 14	< 750	< 540	< 150	< 1250
	nmol/mmol creatinine	< 280	< 45	< 1500	<2732	<496	< 1500
3 - 6 mois	nmol/24h	< 60	< 14	< 750	< 540	< 150	< 1250
	nmol/mmol creatinine	< 280	< 45	< 1500	<1681	<389	< 1500
6-12 mois	nmol/24h	< 60	< 14	< 750	< 540	< 150	< 1250
	nmol/mmol creatinine	< 280	< 45	< 1500	<1164	<268	< 1500
1 - 2 ans	nmol/24h	< 100	< 14	< 1000	< 750	< 320	< 325
	nmol/mmol creatinine	< 80	< 45	< 1300	<593	<216	< 750
2 - 3 ans	nmol/24h	<100	< 19	< 1200	< 750	< 320	< 390
	nmol/mmol creatinine	< 80	< 35	< 740	<456	<213	< 355
3 - 4 ans	nmol/24h	<100	< 19	< 1200	< 750	< 320	< 390
	nmol/mmol creatinine	< 80	< 35	< 740	<456	<213	< 355
4 - 5 ans	nmol/24h	< 170	< 33	< 1200	< 750	< 320	< 390
	nmol/mmol creatinine	< 60	< 20	< 740	<456	<213	< 355
5 - 6 ans	nmol/24h	< 170	< 33	< 1200	< 750	< 320	< 390
	nmol/mmol creatinine	< 60	< 20	< 740	<287	<178	< 355
6 - 7 ans	nmol/24h	< 170	< 33	< 1800	< 1100	< 860	< 1000
	nmol/mmol creatinine	< 60	< 20	< 500	<287	<178	< 355
7 - 8 ans	nmol/24h	< 266	< 55	< 1800	< 1100	< 860	< 1000
	nmol/mmol creatinine	< 60	< 20	< 500	<287	<178	< 355
8 - 9 ans	nmol/24h	< 266	< 55	< 1800	< 1100	< 860	< 1000
	nmol/mmol creatinine	< 60	< 20	< 500	<287	<178	< 355
9 - 10 ans	nmol/24h	< 266	< 55	< 1800	< 1100	< 860	< 1000
	nmol/mmol creatinine	< 60	< 20	< 500	<287	<178	< 355
10 - 16 ans	nmol/24h	< 472	< 110	< 1800	< 1100	< 860	< 1000
	nmol/mmol creatinine	< 55	< 15	< 500	<209	<131	< 355
Adulte	nmol/24h	< 610	< 130	< 3300	< 3800	< 1880	< 1900
	nmol/mmol creatinine	< 45	< 22	< 340	< 250	< 200	< 150

Appendices


Free plasma MN (nmol/l)
1.00
0.10
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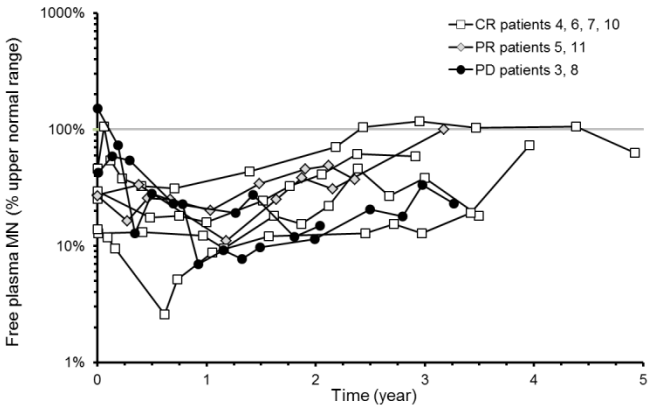
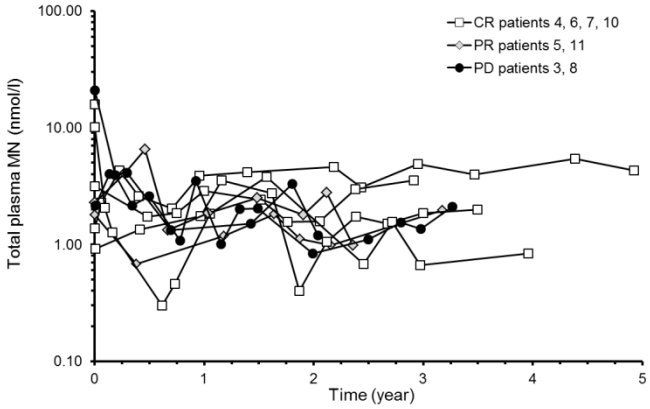
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


Free plasma MN (% upper normal range)
1000%
100%
10%
1%
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


Total plasma MN (nmol/l)
100.00
10.00
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0
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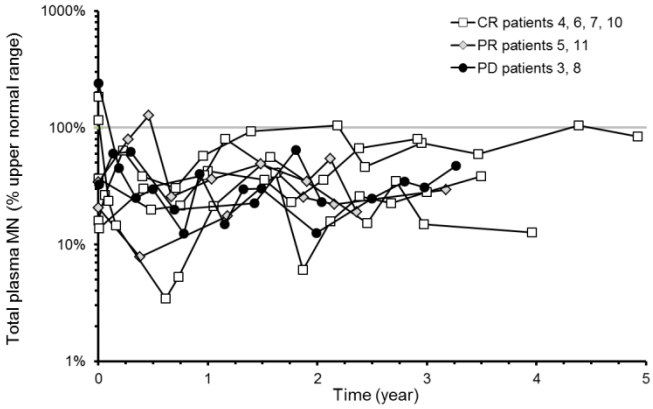
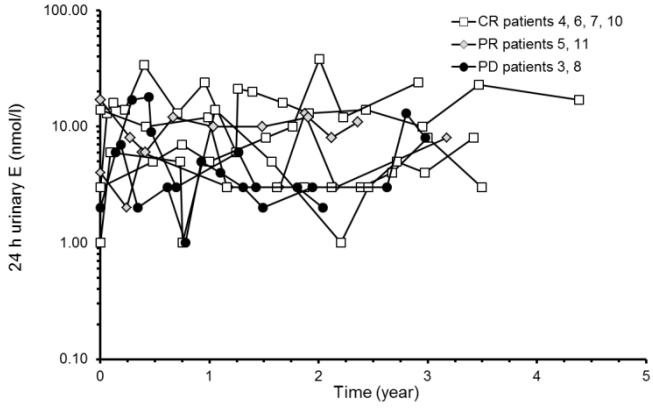
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


Total plasma MN (% upper normal range)
1000%
100%
10%
1%
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


24 h urinary E (nmol/l)
100.00
10.00
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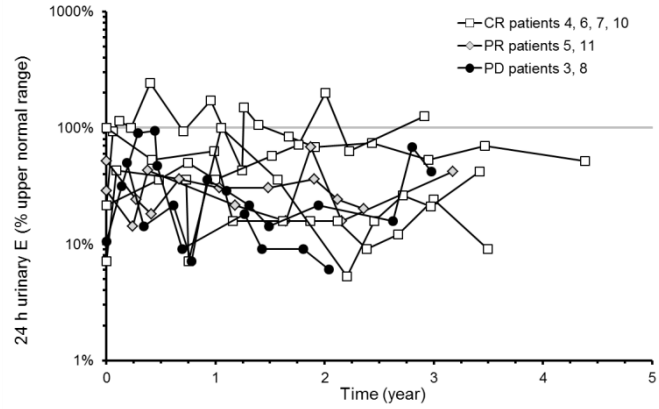
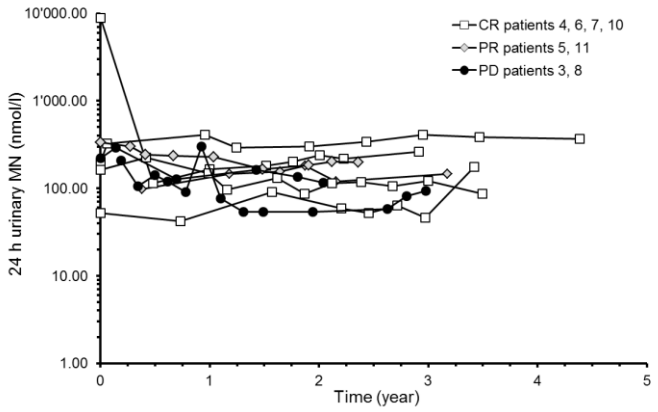
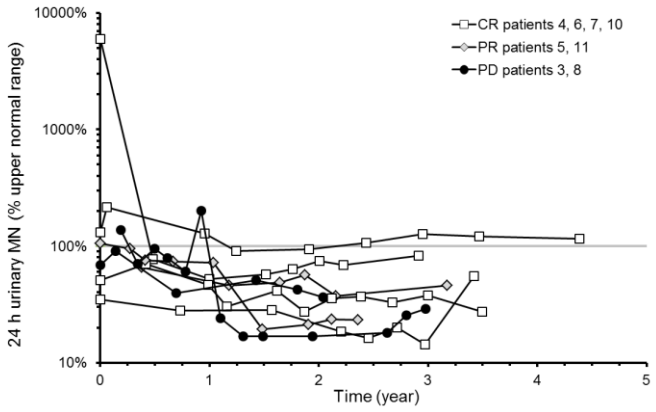
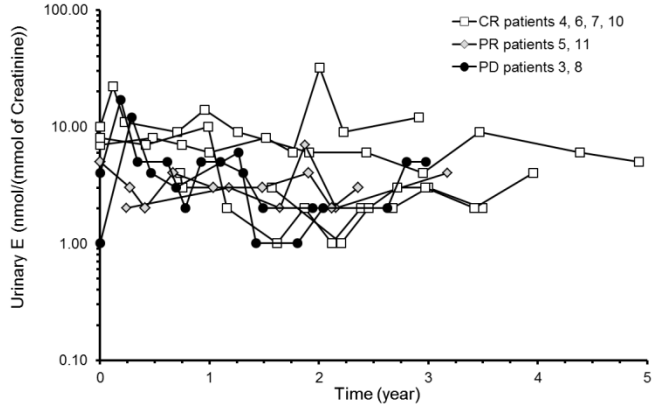
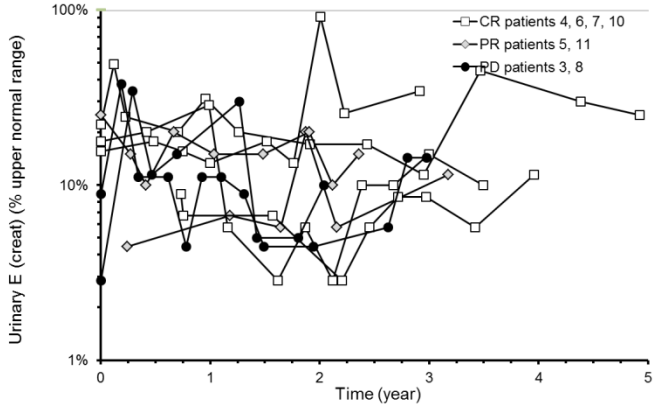
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

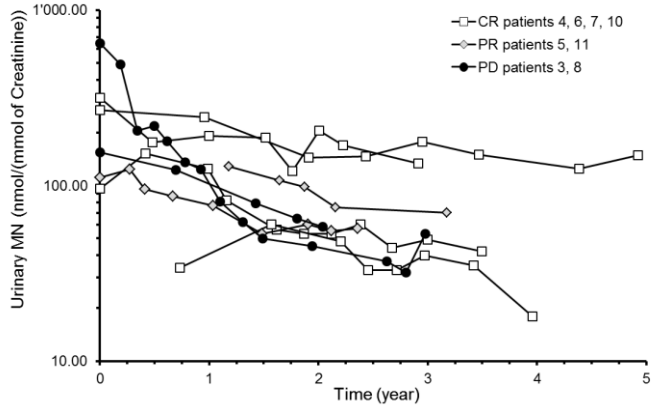

24 h urinary E (% upper normal range)
1000%
100%
10%
1%
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

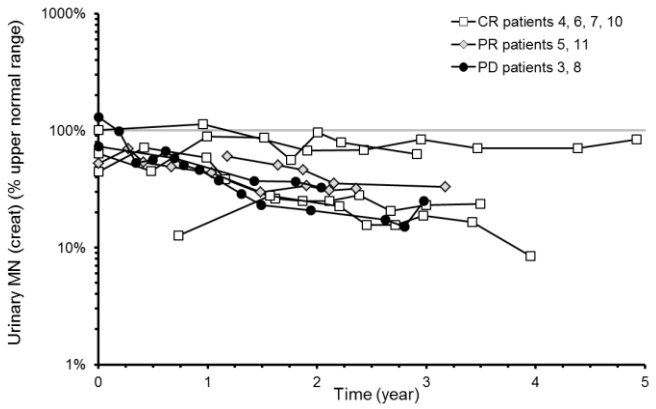

10'000.00
1'000.00
100.00
10.00
1.00
24 h urinary MN (nmol/l)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


10000%
1000%
100%
10%
24 h urinary MN (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


100.00
10.00
1.00
0.10
Urinary E (nmol/(mmol of Creatinine))
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


100%
10%
1%
Urinary E (creat) (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


1'000.00
100.00
10.00
Urinary MN (nmol/(mmol of Creatinine))
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8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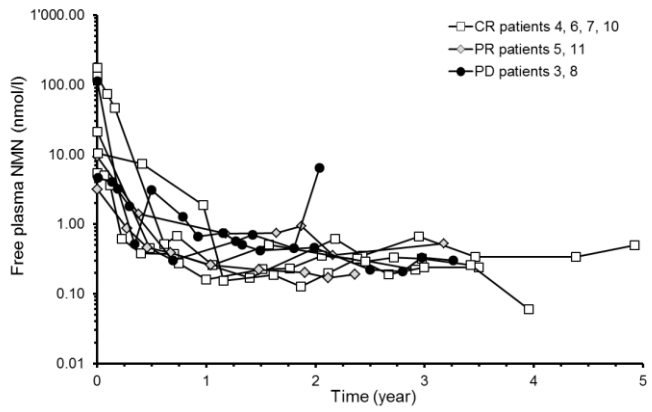
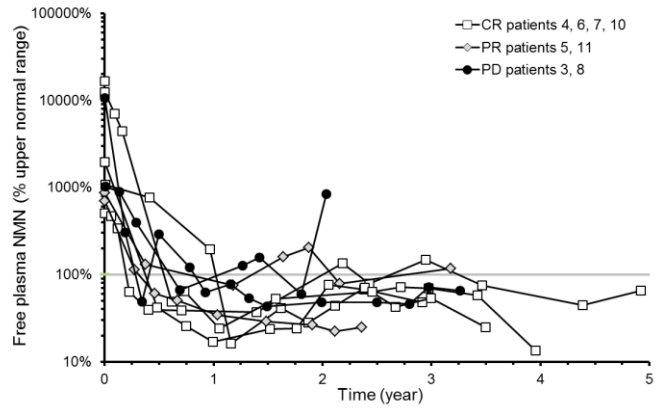
100%
10%
1%
Urinary MN (creat) (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


1'000.00
100.00
10.00
1.00
0.10
0.01
Free plasma NMN (nmol/l)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8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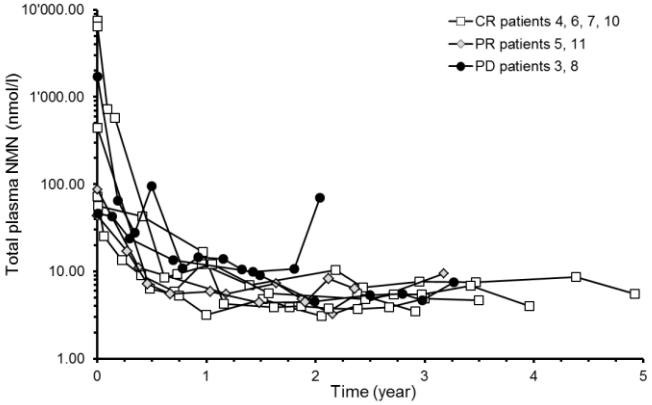
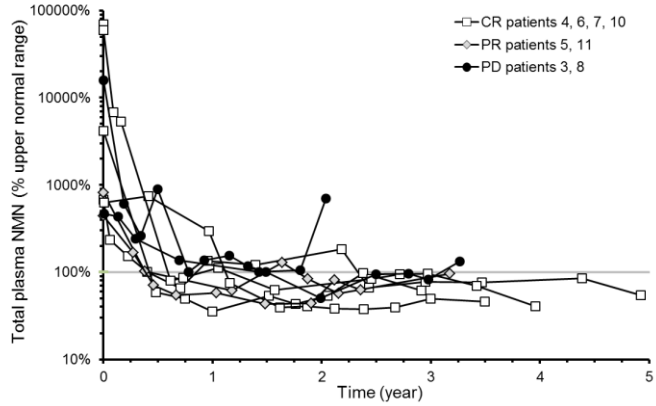
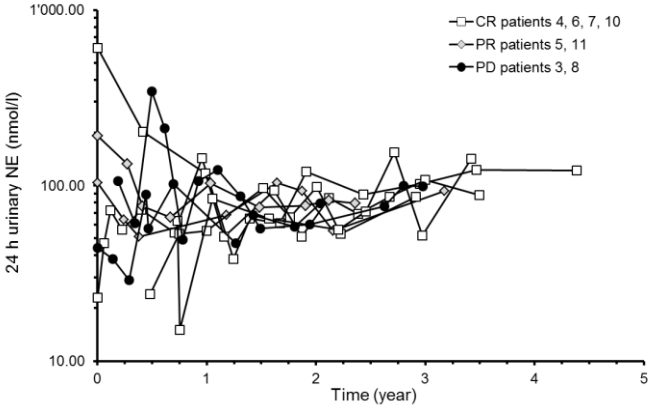
10000%
1000%
100%
10%
Free plasma NMN (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


10'000.00
1'000.00
100.00
10.00
1.00
Total plasma NMN (nmol/l)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8


100000%
10000%
1000%
100%
10%
Total plasma NMN (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8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10.00
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1
2
3
4
5
Time (year)
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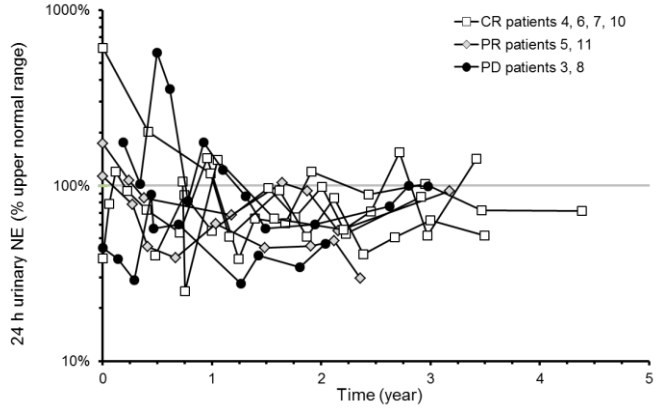
PR patients 5, 11
PD patients 3, 8


1000%
100%
10%
24 h urinary NE (% upper normal range)
0
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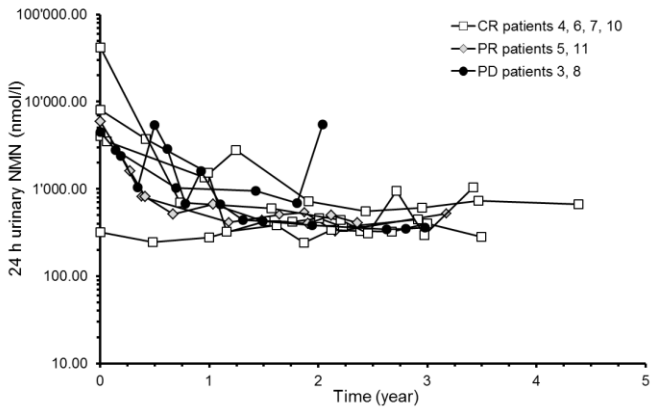
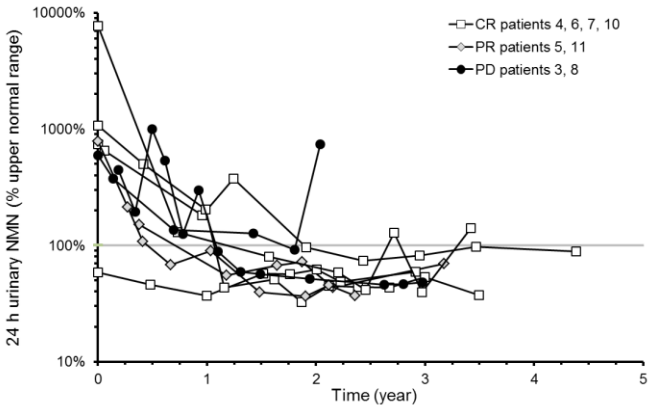
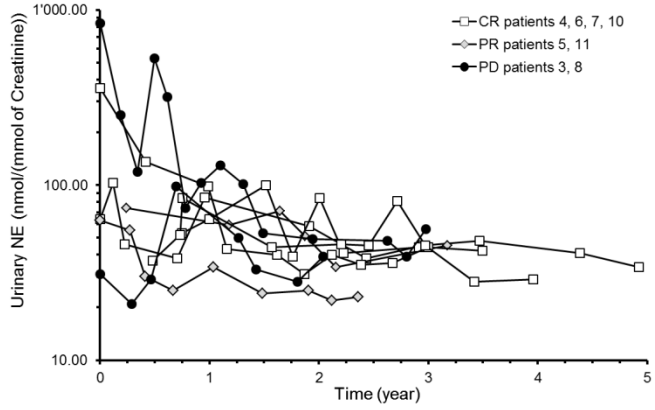
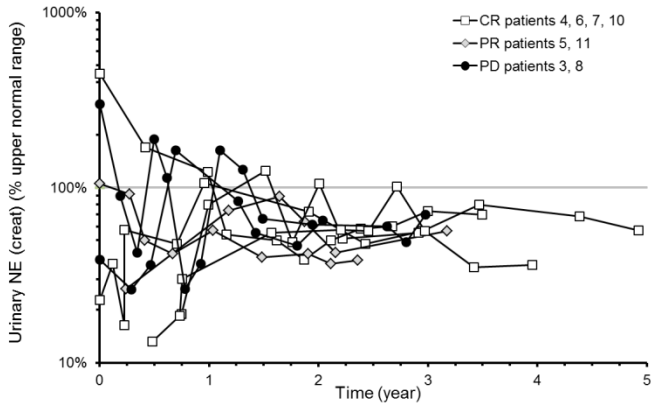
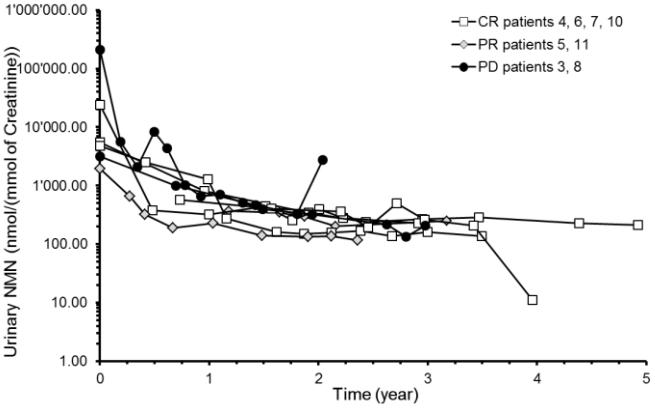
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

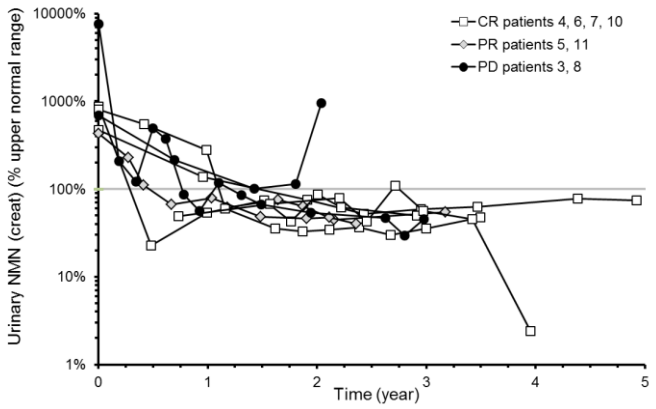

CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
24 h urinary NMN (nmol/l)
100'000.00
10'000.00
1'000.00
100.00
10.00
0
1
2
3
4
5
Time (year)

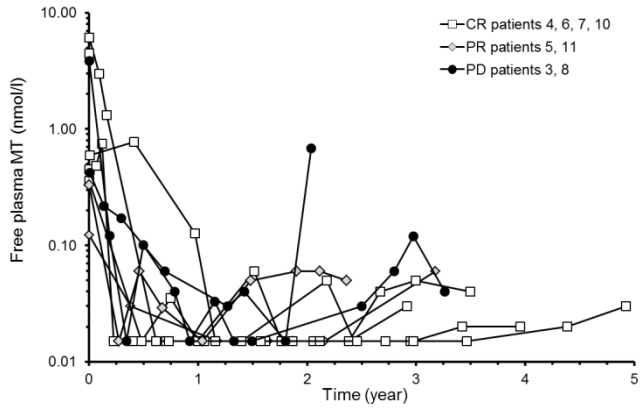

CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
24 h urinary NMN (% upper normal range)
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Urinary NE (nmol/(mmol of Creatinine))
1'000.00
100.00
10.00
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Urinary NE (creat) (% upper normal range)
1000%
100%
10%
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Urinary NMN (nmol/(mmol of Creatinine))
1'000'000.00
100'000.00
10'000.00
1'000.00
100.00
10.00
1.00
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Urinary NMN (creat) (% upper normal range)
10000%
1000%
100%
10%
1%
0
1
2
3
4
5
Time (year)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PR patients 5, 11
PD patients 3, 8
Free plasma MT (nmol/l)
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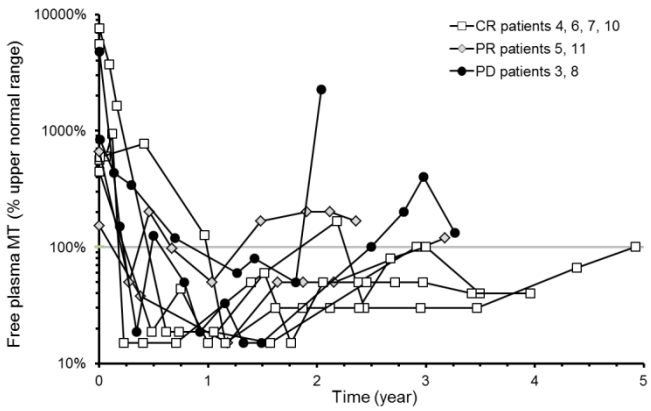
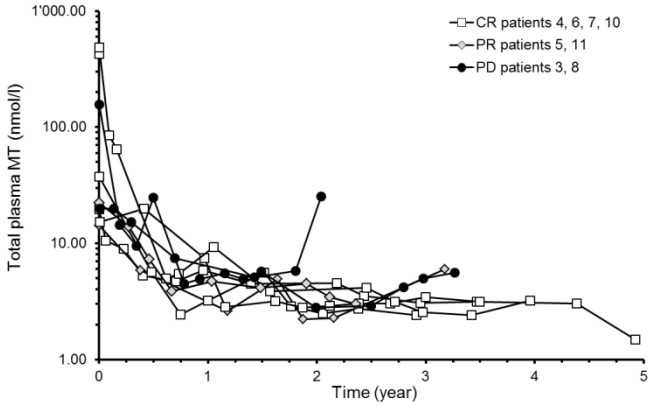
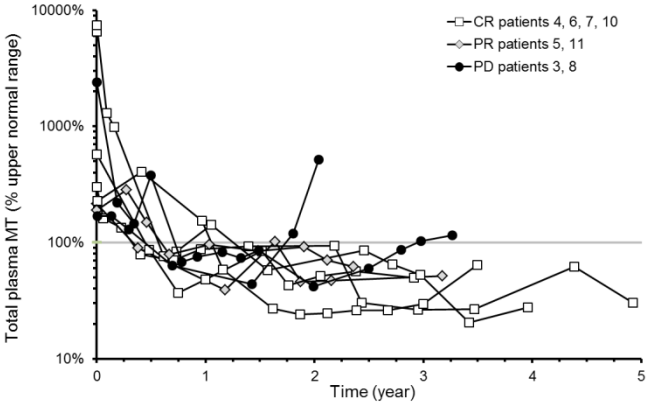
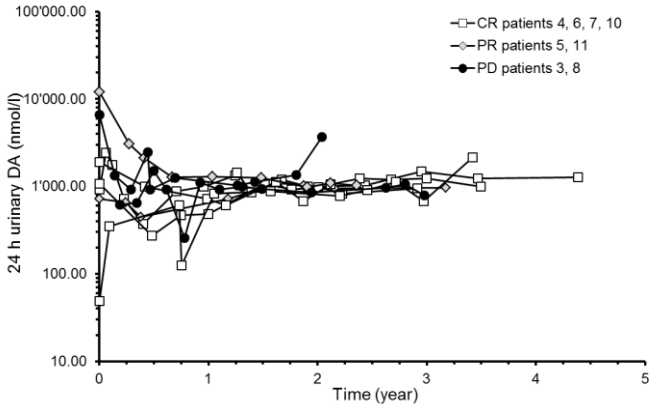
0.01
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Free plasma MT (% upper normal range)
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Total plasma MT (nmol/l)
1'000.00
100.00
10.00
1.00
0
1
2
3
4
5
Time (year)


CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
Total plasma MT (% upper normal range)
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)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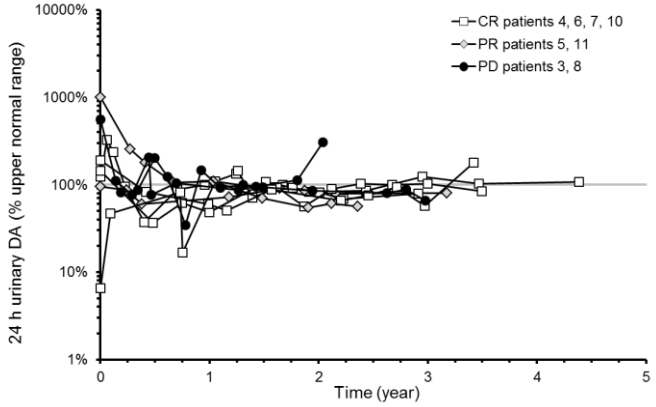
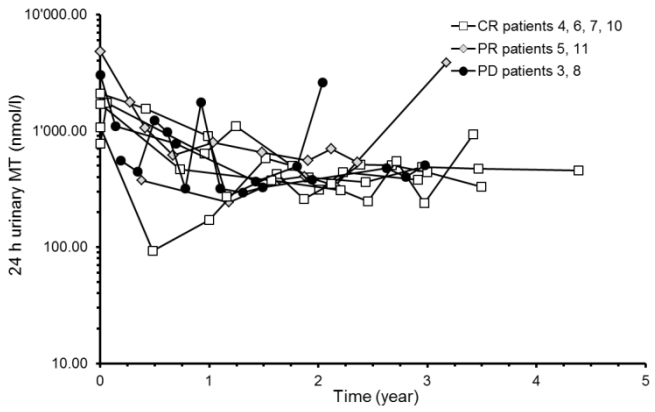
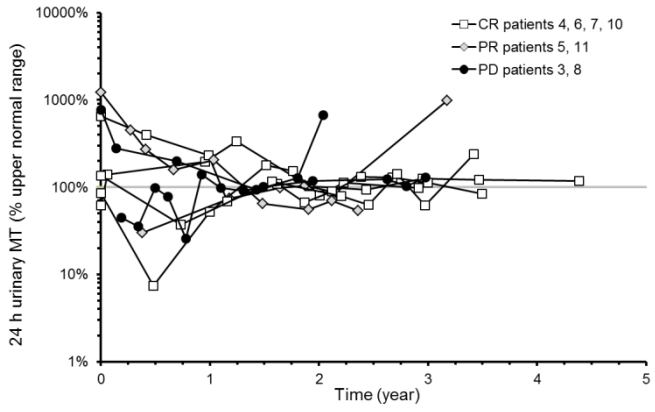
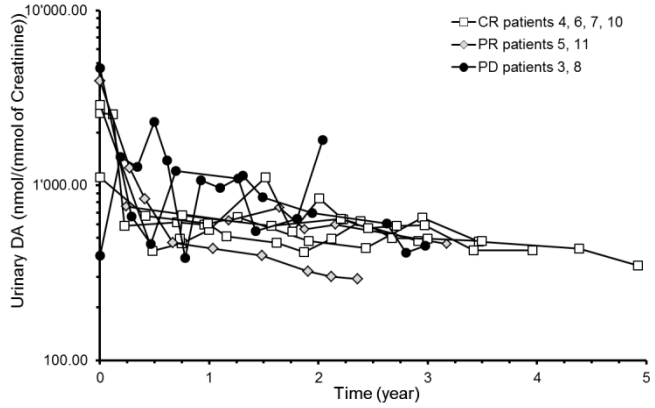
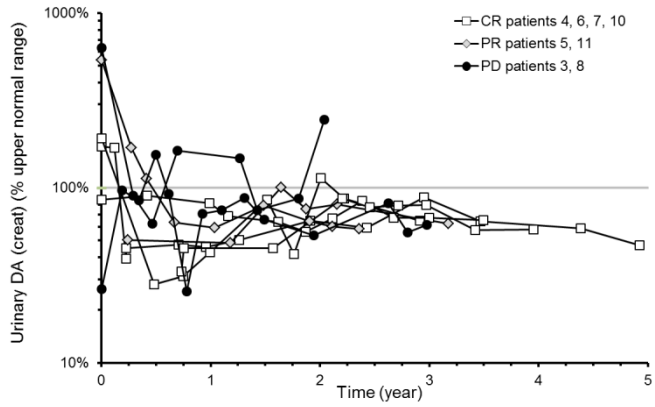
PR patients 5, 11
PD patients 3, 8
24 h urinary DA (nmol/l)
100'000.00
10'000.00
1'000.00
100.00
10.00
0
1
2
3
4
5
Time (year)

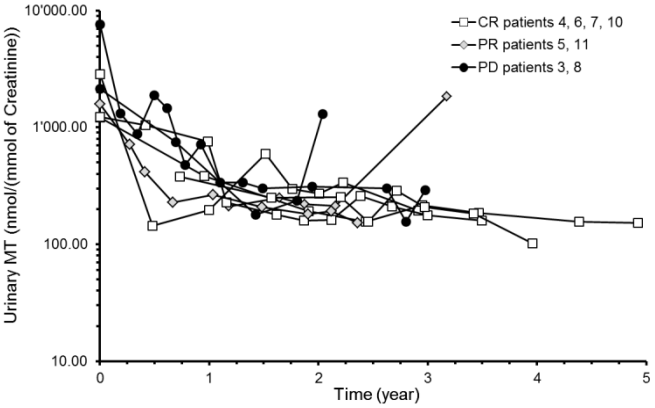

CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8
24 h urinary DA (% upper normal range)
10000%
1000%
100%
10%
1%
0
1
2
3
4
5
Time (year)

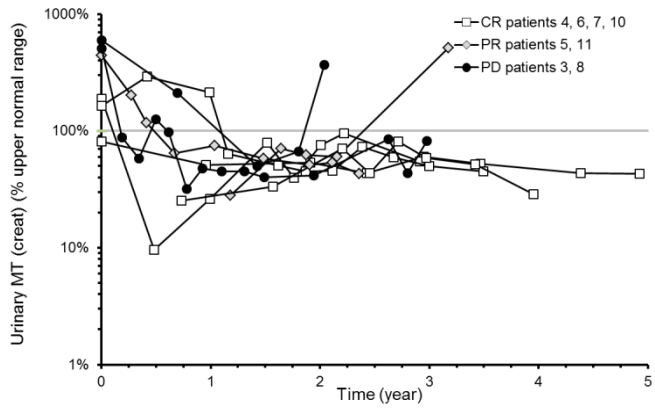

10'000.00
1'000.00
100.00
10.00
24 h urinary MT (nmol/l)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

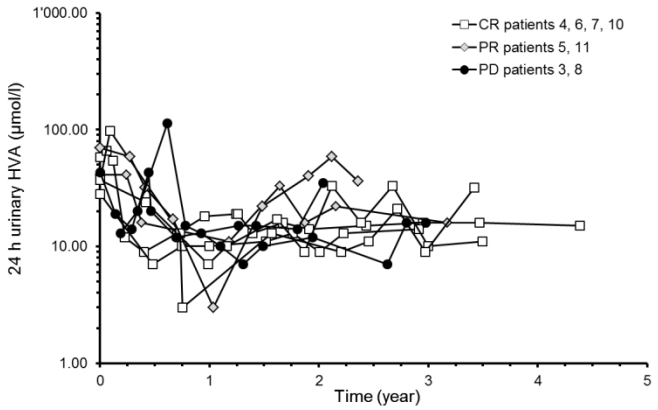

10000%
1000%
100%
10%
1%
24 h urinary MT (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

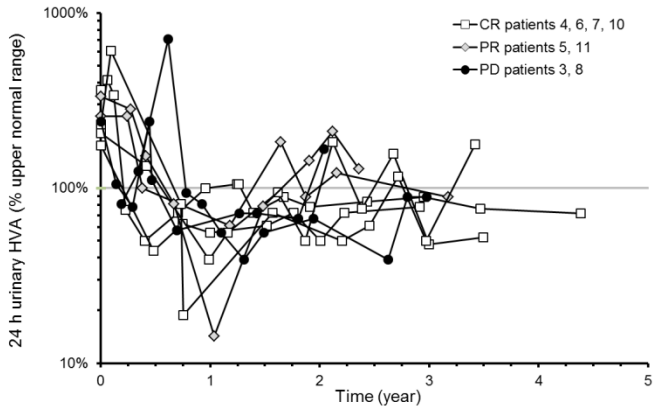

10'000.00
1'000.00
100.00
Urinary DA (nmol/(mmol of Creatinine))
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

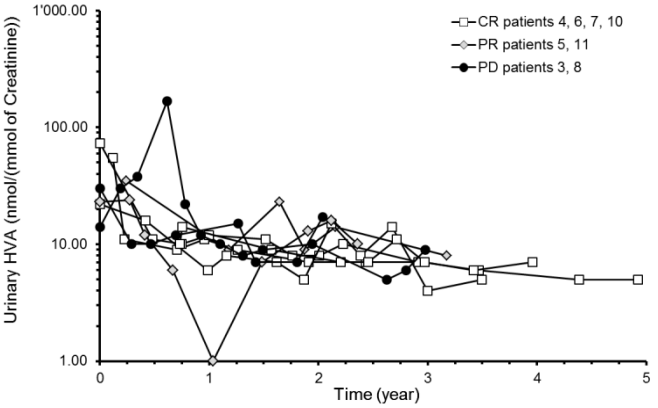

1000%
100%
10%
Urinary DA (creat) (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

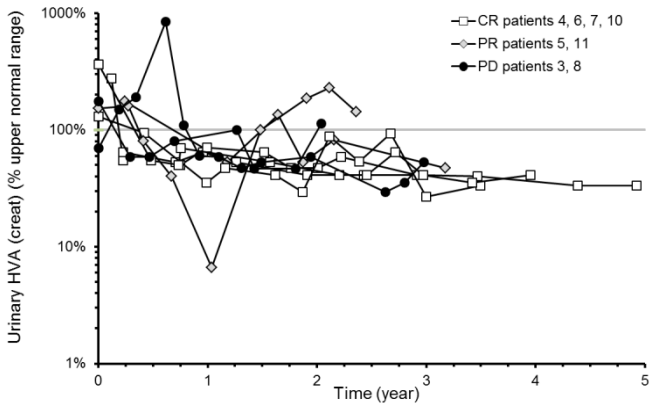

10'000.00
1'000.00
100.00
10.00
Urinary MT (nmol/(mmol of Creatinine))
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

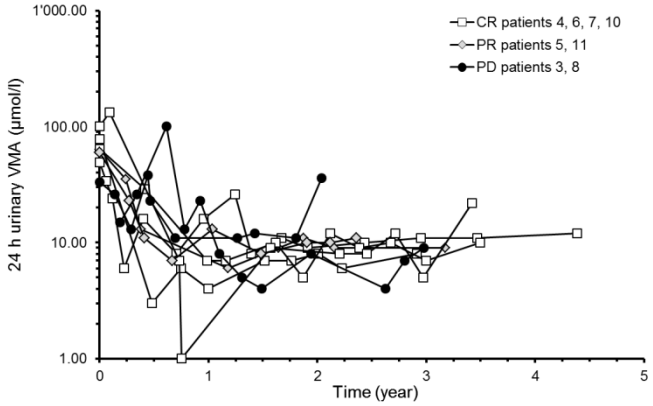

1000%
100%
10%
1%
Urinary MT (creat) (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

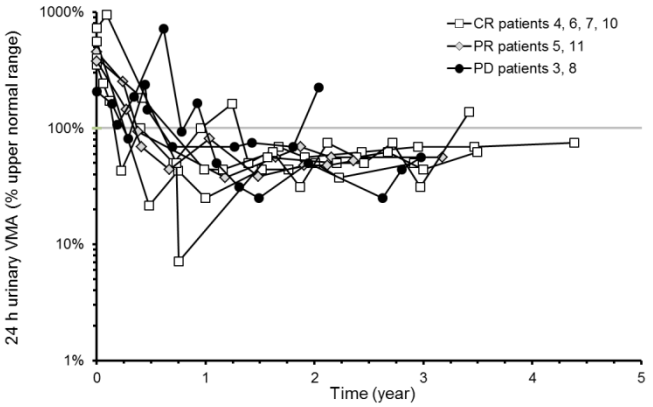

1'000.00
100.00
10.00
1.00
24 h urinary HVA (µmol/l)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

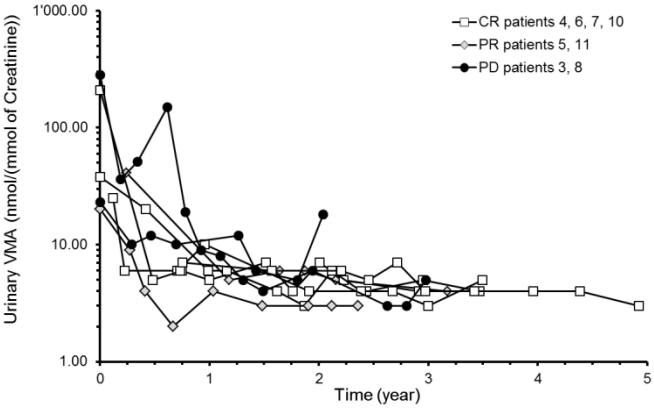

1000%
100%
10%
24 h urinary HVA (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

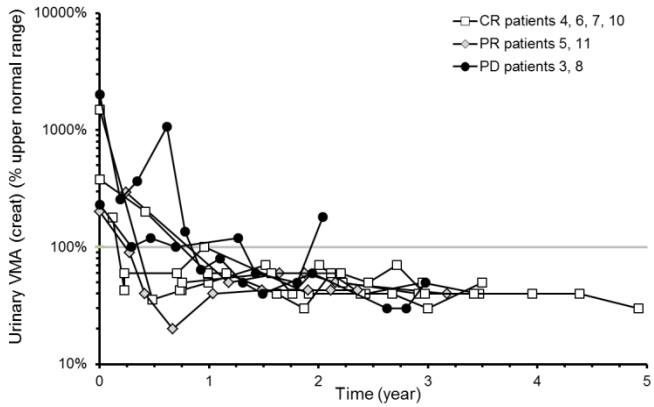

1'000.00
100.00
10.00
1.00
Urinary HVA (nmol/(mmol of Creatinine))
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

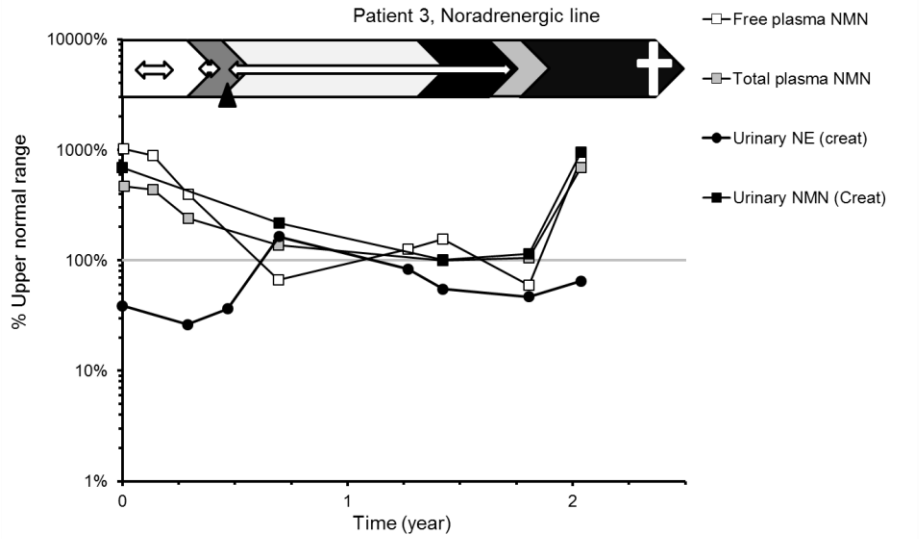

1000%
100%
10%
1%
Urinary HVA (creat) (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

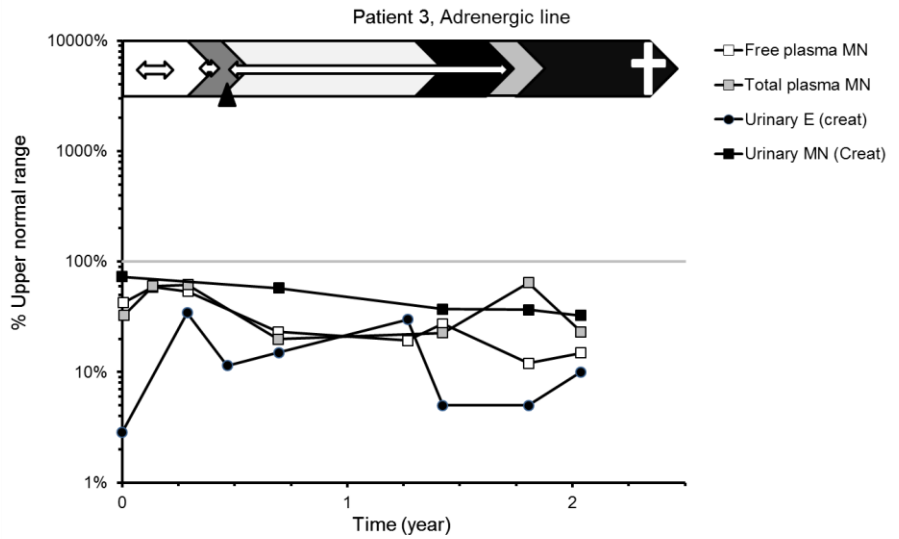

1'000.00
100.00
10.00
1.00
24 h urinary VMA (µmol/l)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

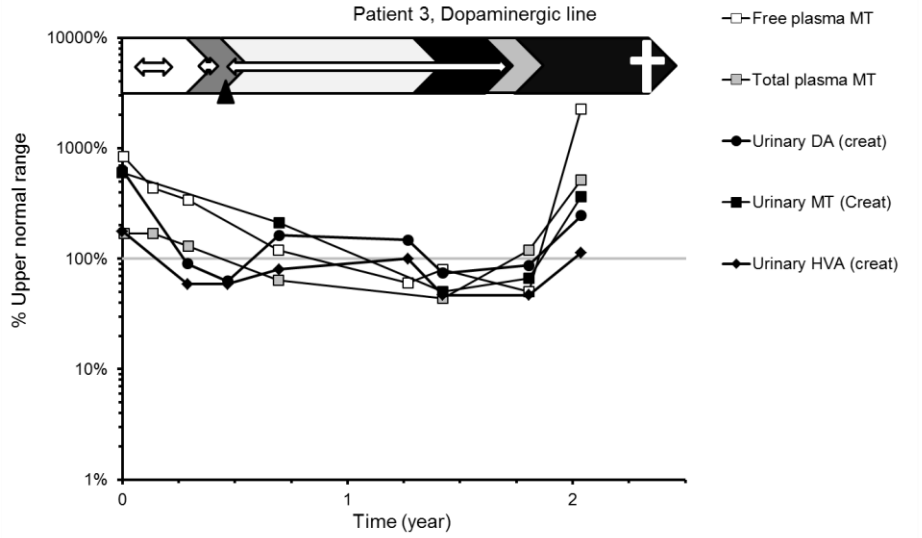

1000%
100%
10%
1%
24 h urinary VMA (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

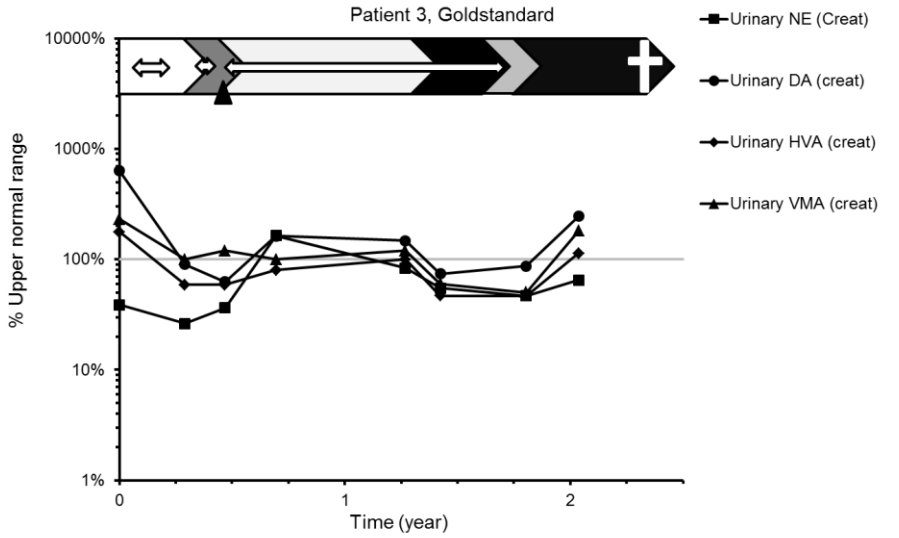

1'000.00
100.00
10.00
1.00
Urinary VMA (nmol/(mmol of Creatinine))
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

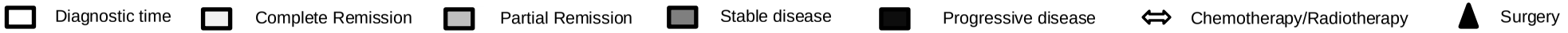

10000%
1000%
100%
10%
Urinary VMA (creat) (% upper normal range)
0
1
2
3
4
5
Time (year)
CR patients 4, 6, 7, 10
PR patients 5, 11
PD patients 3, 8

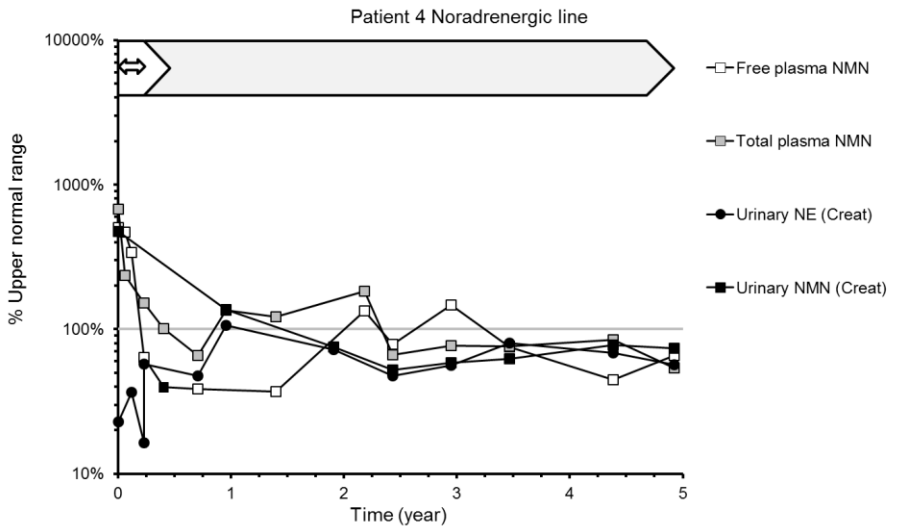

Patient 3, Noradrenergic line
Free plasma NMN
Total plasma NMN
Urinary NE (creat)
Urinary NMN (Creat)
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)

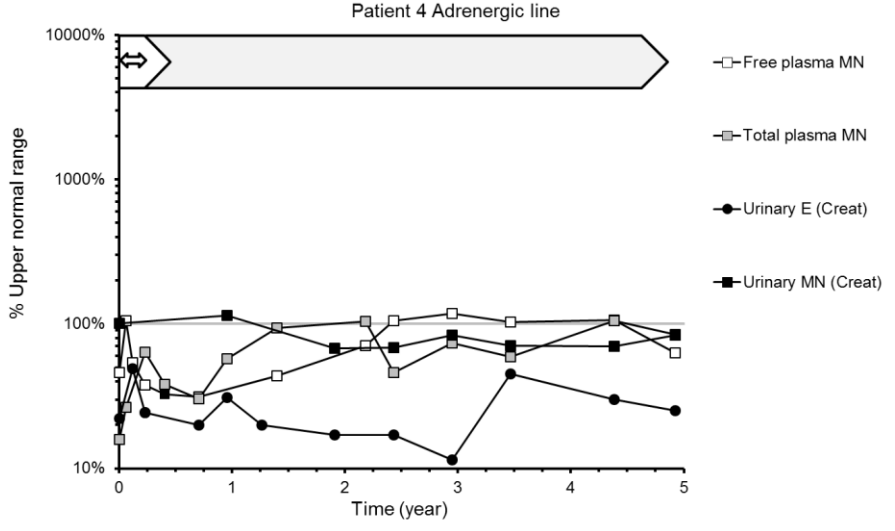

Patient 3, Adrenergic line
Free plasma MN
Total plasma MN
Urinary E (creat)
Urinary MN (Creat)
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)

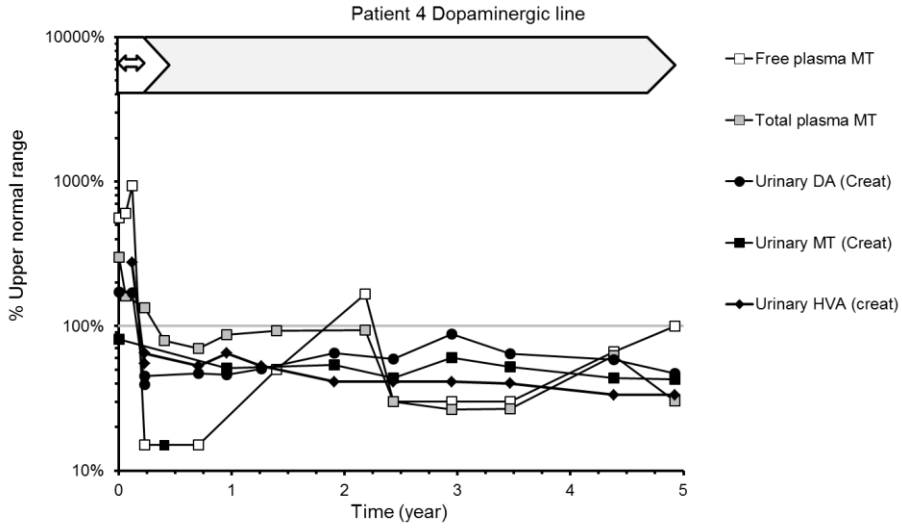

Patient 3, Dopaminergic line
Free plasma MT
Total plasma MT
Urinary DA (creat)
Urinary MT (Creat)
Urinary HVA (creat)
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)

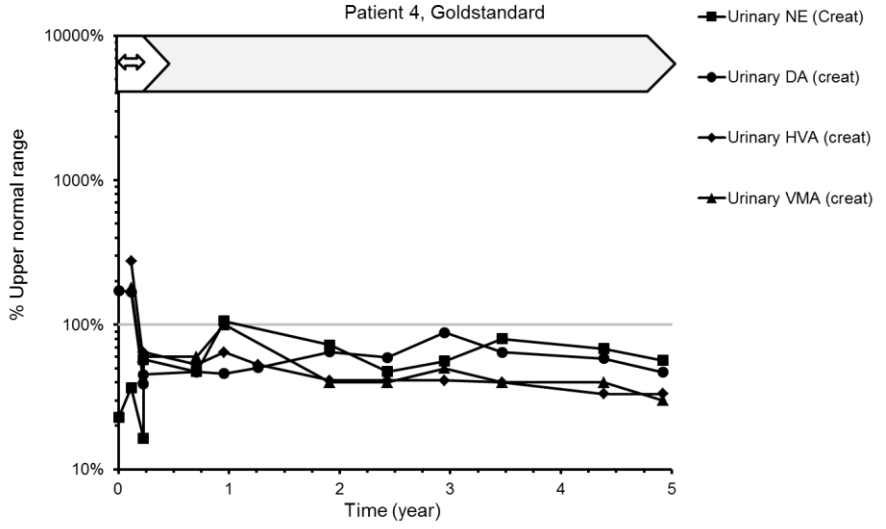

Patient 3, Goldstandard
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)

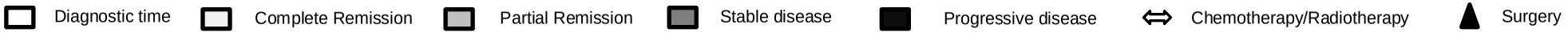

Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery

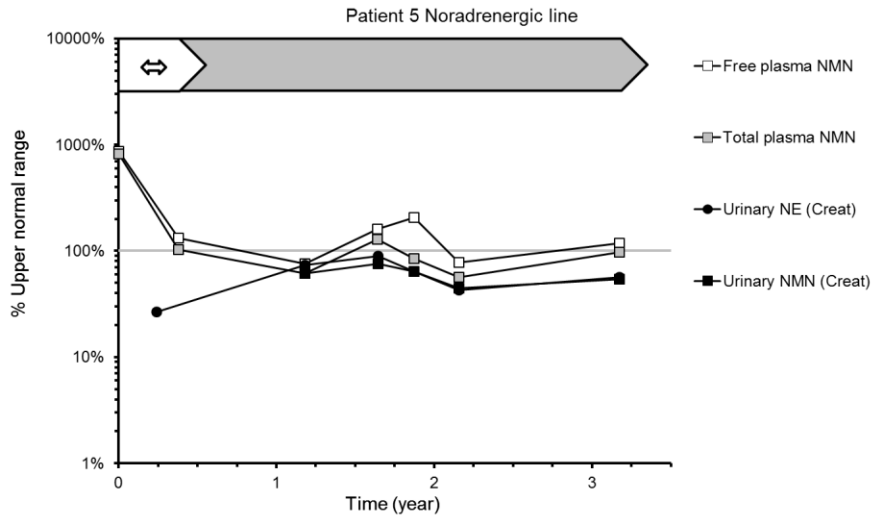

Patient 4 Noradrenergic line
% Upper normal range
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)
Free plasma NMN
Total plasma NMN
Urinary NE (Creat)
Urinary NMN (Creat)

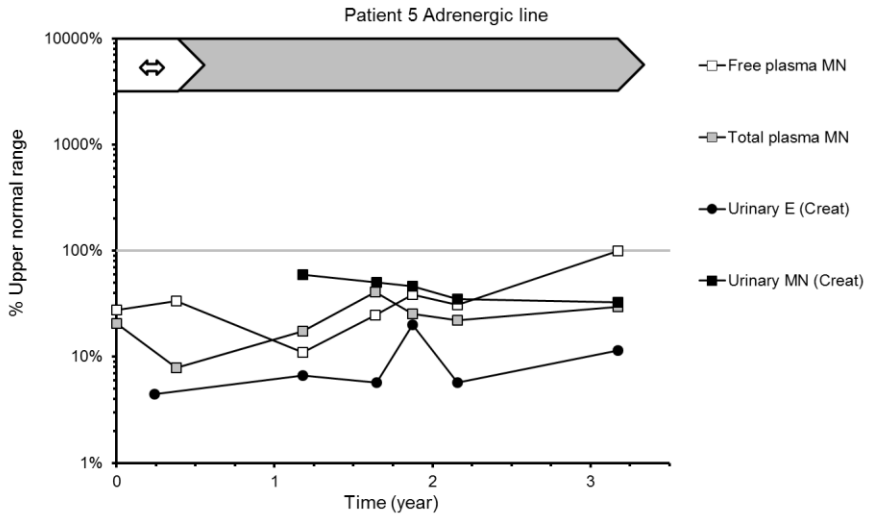

Patient 4 Adrenergic line
% Upper normal range
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)
Free plasma MN
Total plasma MN
Urinary E (Creat)
Urinary MN (Creat)

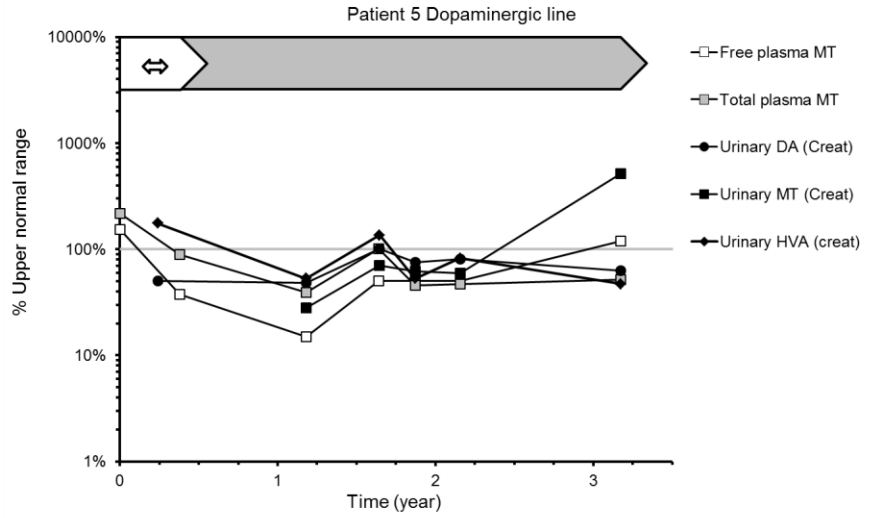

Patient 4 Dopaminergic line
% Upper normal range
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)
Free plasma MT
Total plasma MT
Urinary DA (Creat)
Urinary MT (Creat)
Urinary HVA (creat)

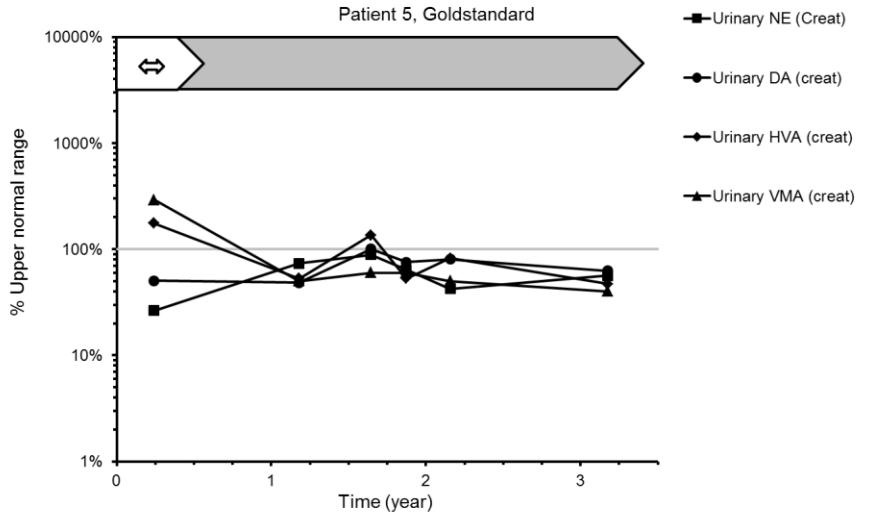

Patient 4, Goldstandard
% Upper normal range
10000%
1000%
100%
10%
0
1
2
3
4
5
Time (year)
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)

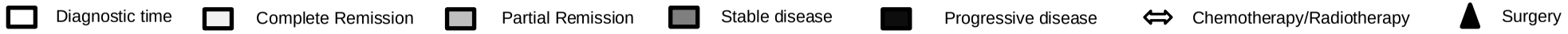

Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery

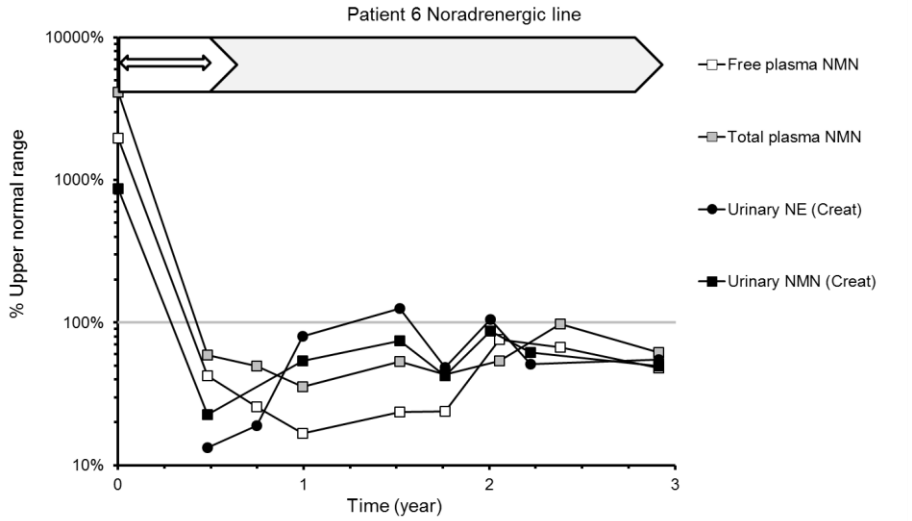

Patient 5 Noradrenergic line
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
Time (year)
Free plasma NMN
Total plasma NMN
Urinary NE (Creat)
Urinary NMN (Creat)

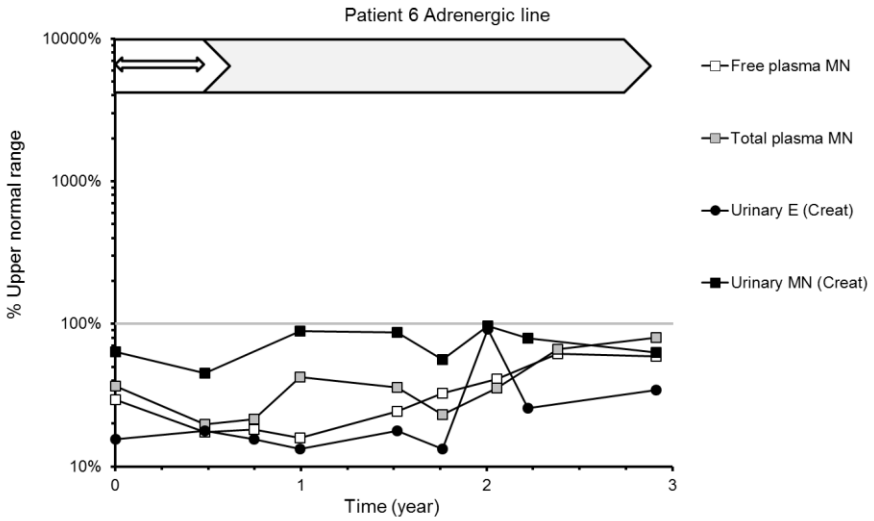

Patient 5 Adrenergic line
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
Time (year)
Free plasma MN
Total plasma MN
Urinary E (Creat)
Urinary MN (Creat)

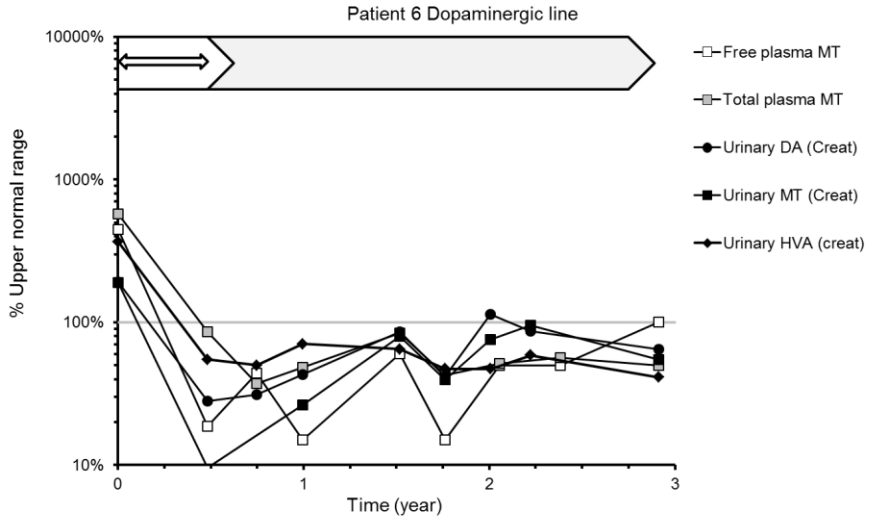

Patient 5 Dopaminergic line
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
Time (year)
Free plasma MT
Total plasma MT
Urinary DA (Creat)
Urinary MT (Creat)
Urinary HVA (creat)

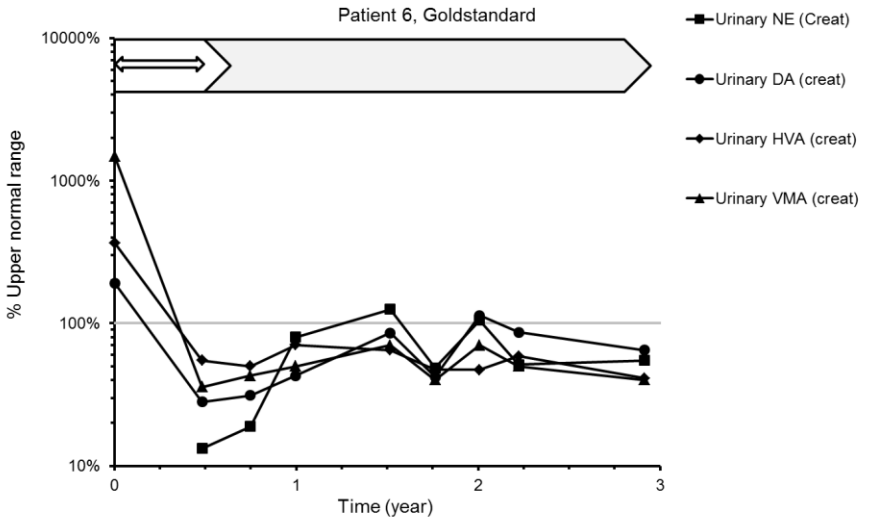

Patient 5, Goldstandard
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
Time (year)
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)

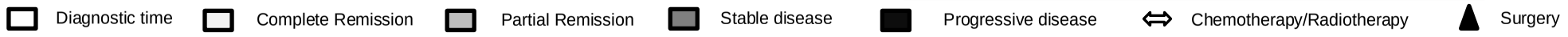

Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery

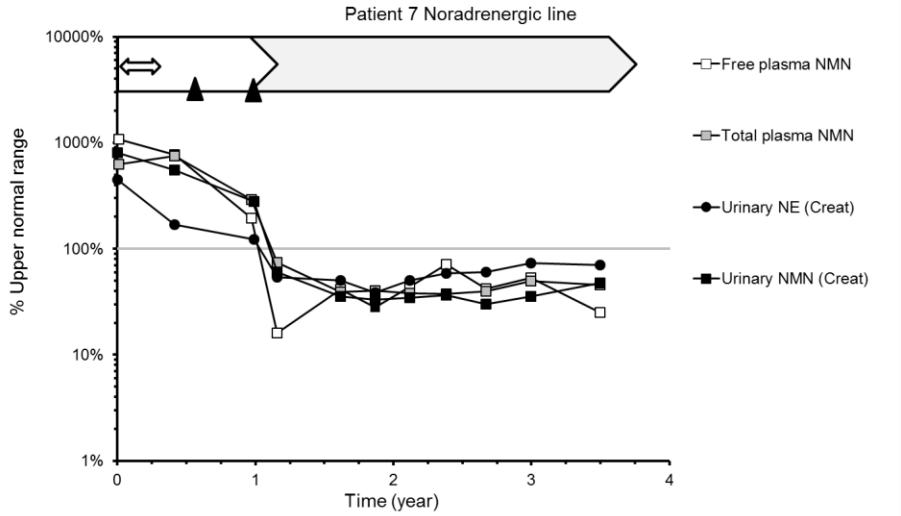

Patient 6 Noradrenergic line
10000%
1000%
100%
10%
% Upper normal range
0
1
2
3
Time (year)
Free plasma NMN
Total plasma NMN
Urinary NE (Creat)
Urinary NMN (Creat)

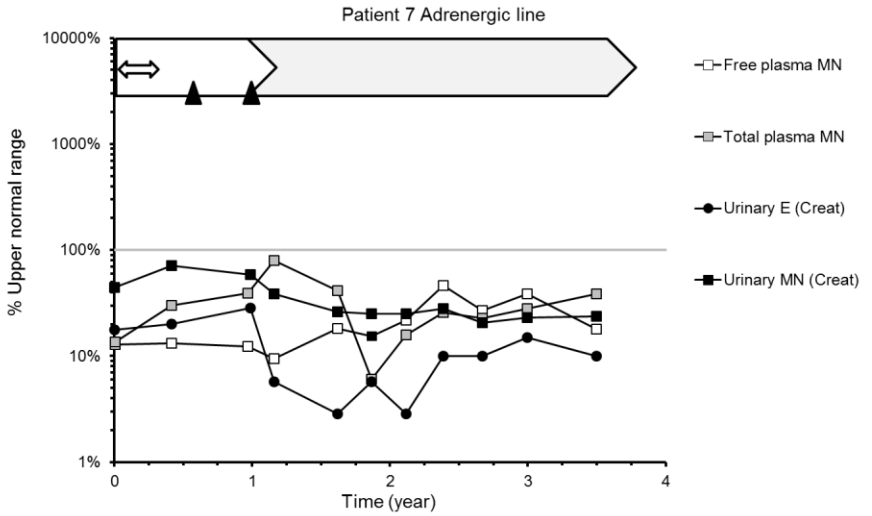

Patient 6 Adrenergic line
10000%
1000%
100%
10%
% Upper normal range
0
1
2
3
Time (year)
Free plasma MN
Total plasma MN
Urinary E (Creat)
Urinary MN (Creat)

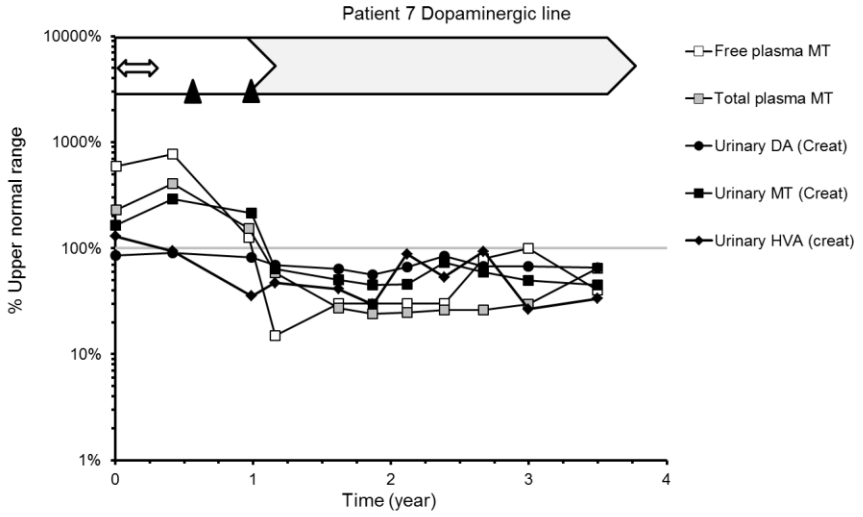

Patient 6 Dopaminergic line
10000%
1000%
100%
10%
% Upper normal range
0
1
2
3
Time (year)
Free plasma MT
Total plasma MT
Urinary DA (Creat)
Urinary MT (Creat)
Urinary HVA (creat)

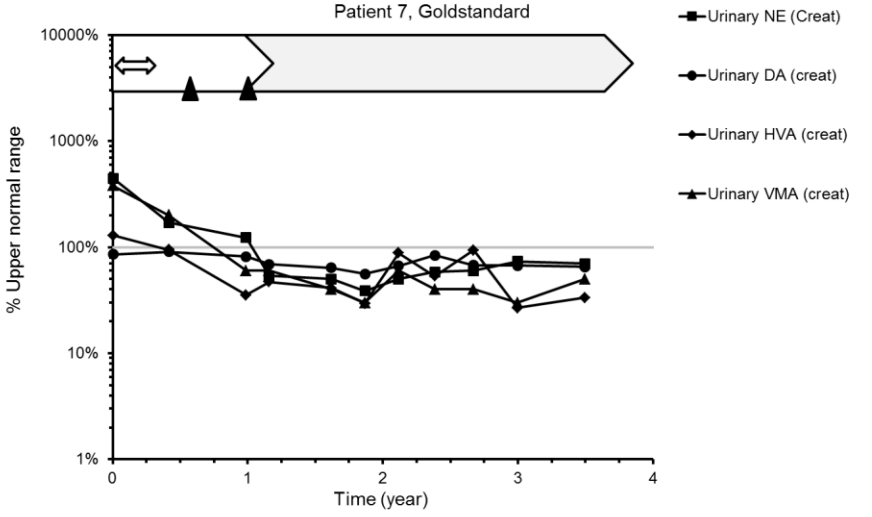

Patient 6, Goldstandard
10000%
1000%
100%
10%
% Upper normal range
0
1
2
3
Time (year)
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)

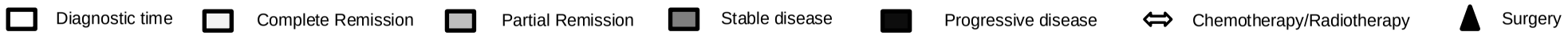

Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery

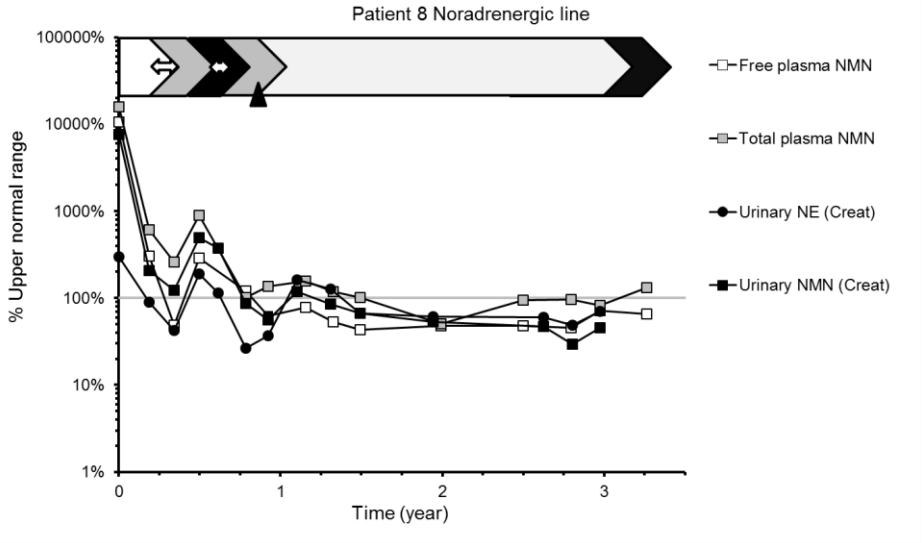

Patient 7 Noradrenergic line
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
4
Time (year)
Free plasma NMN
Total plasma NMN
Urinary NE (Creat)
Urinary NMN (Creat)

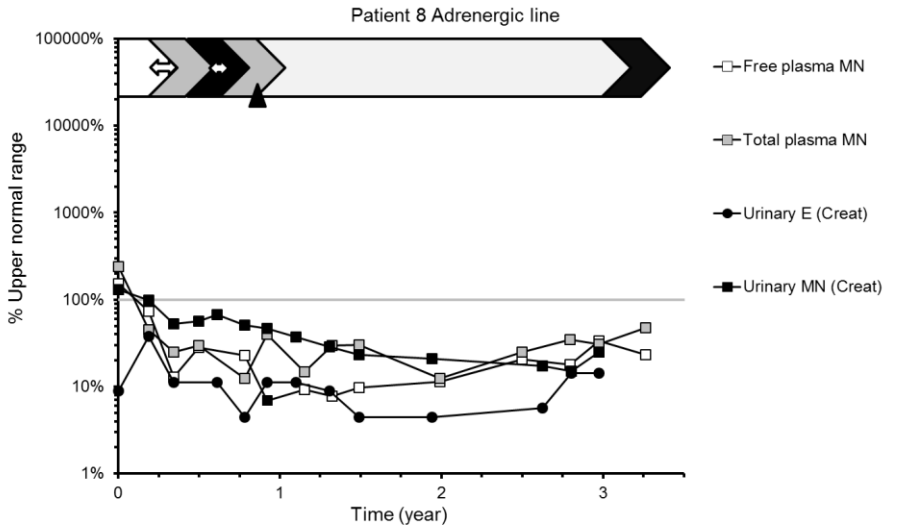

Patient 7 Adrenergic line
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
4
Time (year)
Free plasma MN
Total plasma MN
Urinary E (Creat)
Urinary MN (Creat)

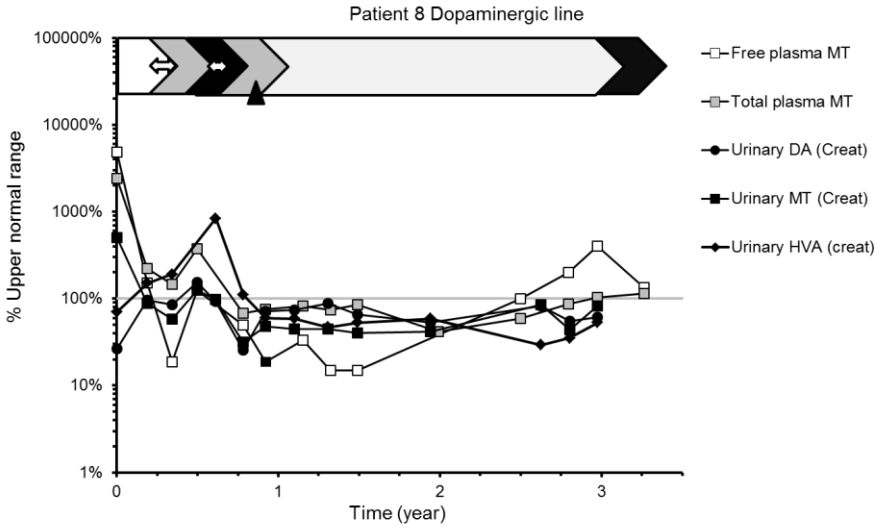

Patient 7 Dopaminergic line
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
4
Time (year)
Free plasma MT
Total plasma MT
Urinary DA (Creat)
Urinary MT (Creat)
Urinary HVA (creat)

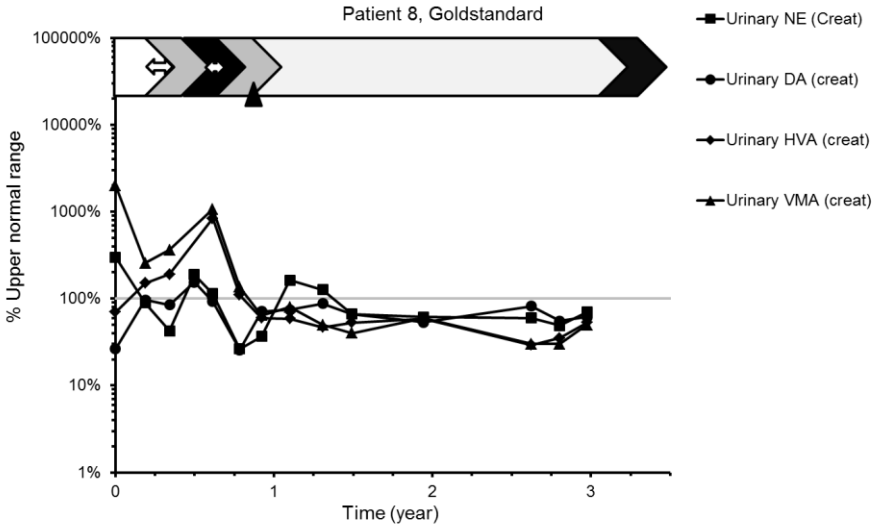

Patient 7, Goldstandard
10000%
1000%
100%
10%
1%
% Upper normal range
0
1
2
3
4
Time (year)
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)

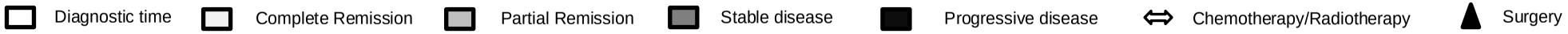

Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery

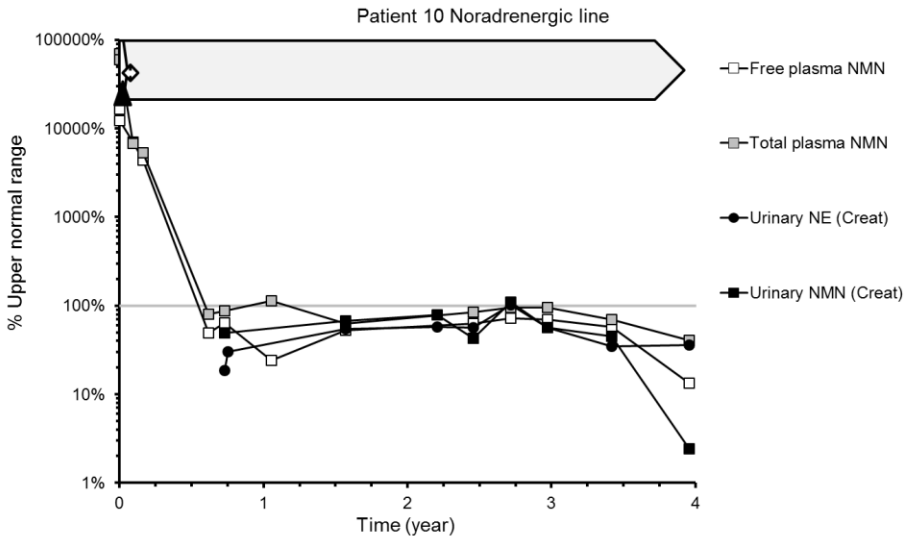

Patient 8 Noradrenergic line
% Upper normal range
100000%
10000%
1000%
100%
10%
1%
0
1
2
3
Time (year)
Free plasma NMN
Total plasma NMN
Urinary NE (Creat)
Urinary NMN (Creat)

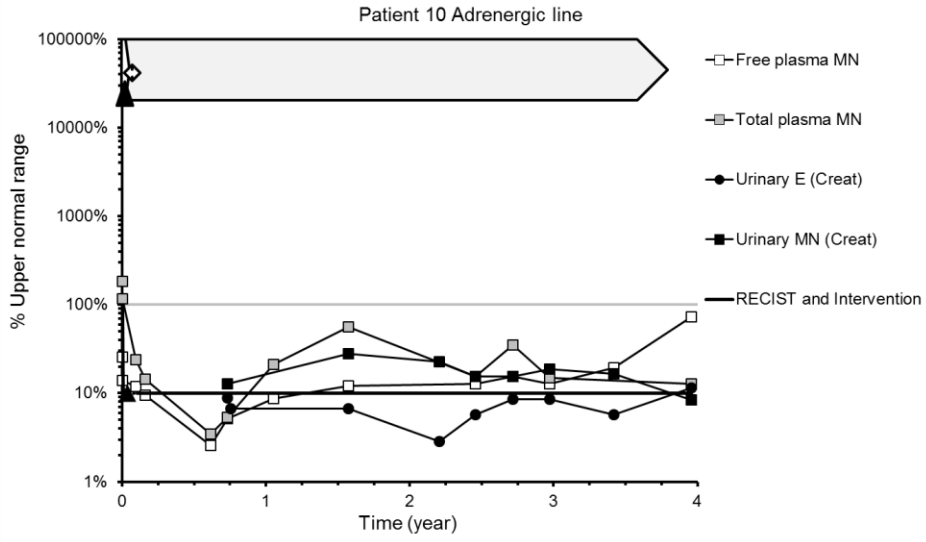

Patient 8 Adrenergic line
% Upper normal range
100000%
10000%
1000%
100%
10%
1%
0
1
2
3
Time (year)
Free plasma MN
Total plasma MN
Urinary E (Creat)
Urinary MN (Creat)

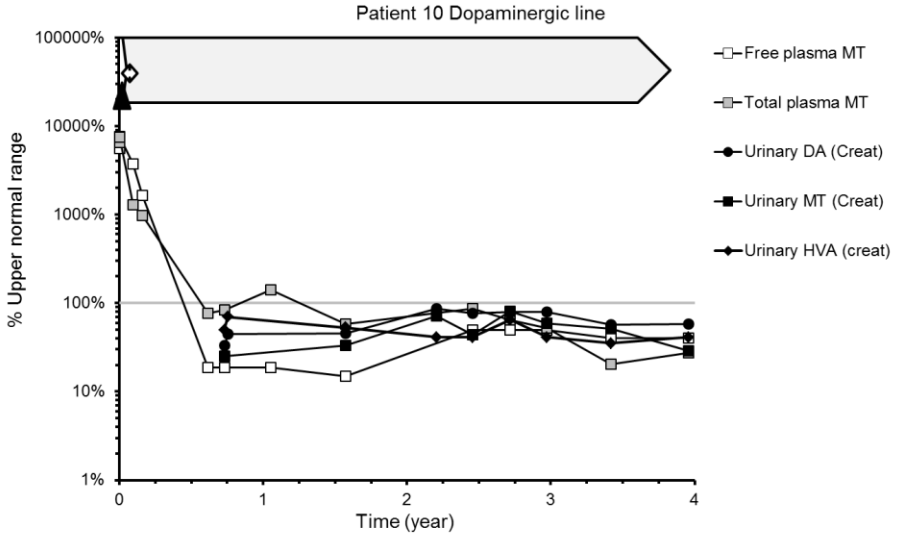

Patient 8 Dopaminergic line
% Upper normal range
100000%
10000%
1000%
100%
10%
1%
0
1
2
3
Time (year)
Free plasma MT
Total plasma MT
Urinary DA (Creat)
Urinary MT (Creat)
Urinary HVA (creat)

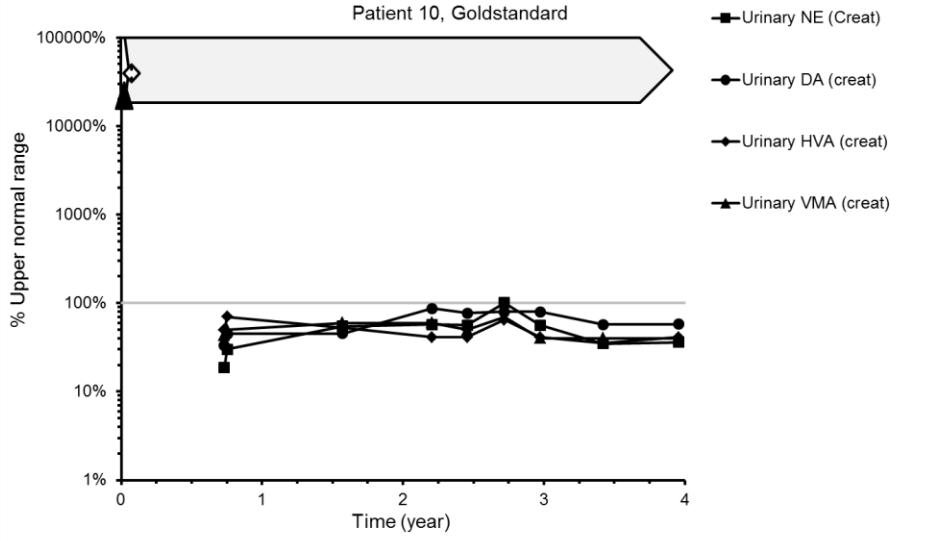

Patient 8, Goldstandard
% Upper normal range
100000%
10000%
1000%
100%
10%
1%
0
1
2
3
Time (year)
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)


Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery

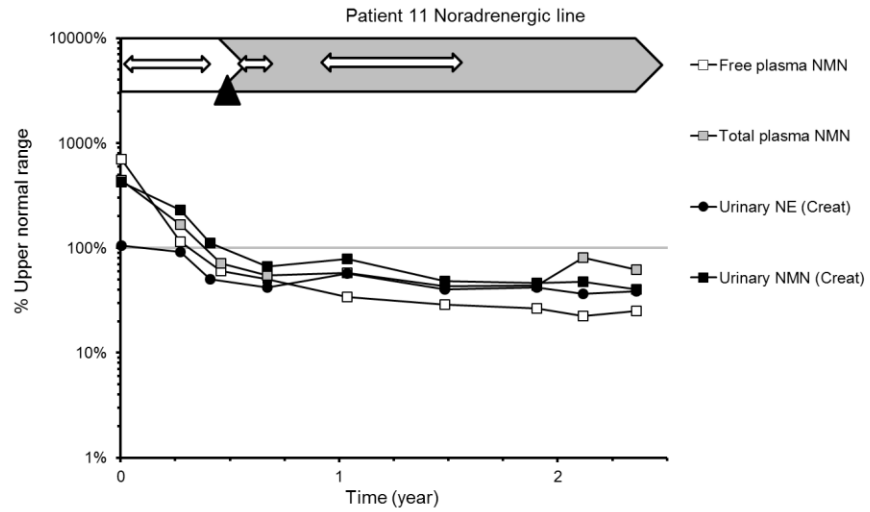


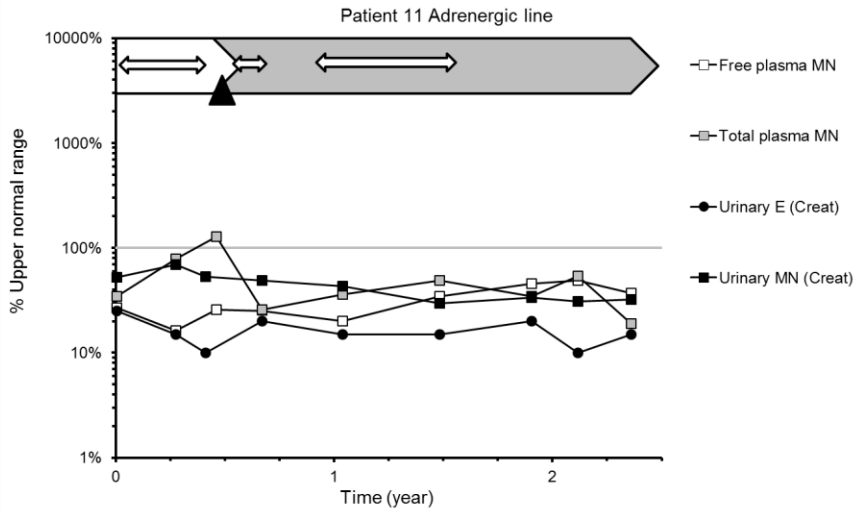


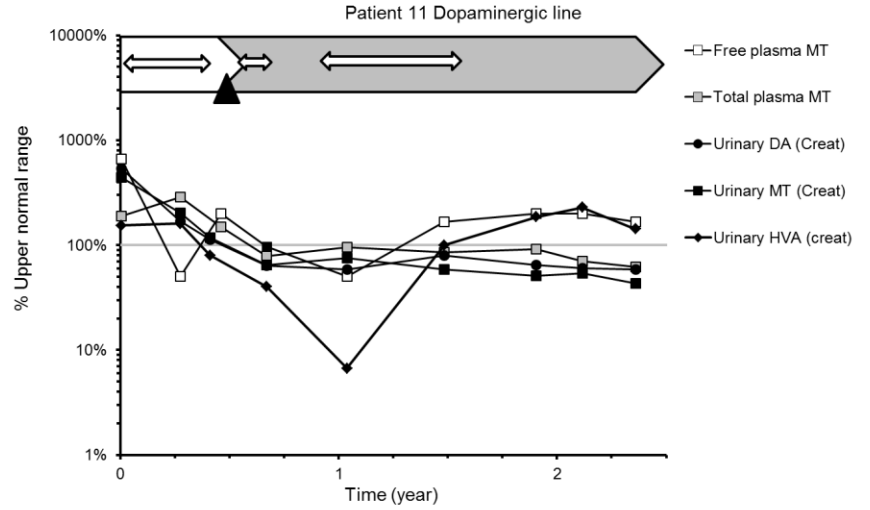


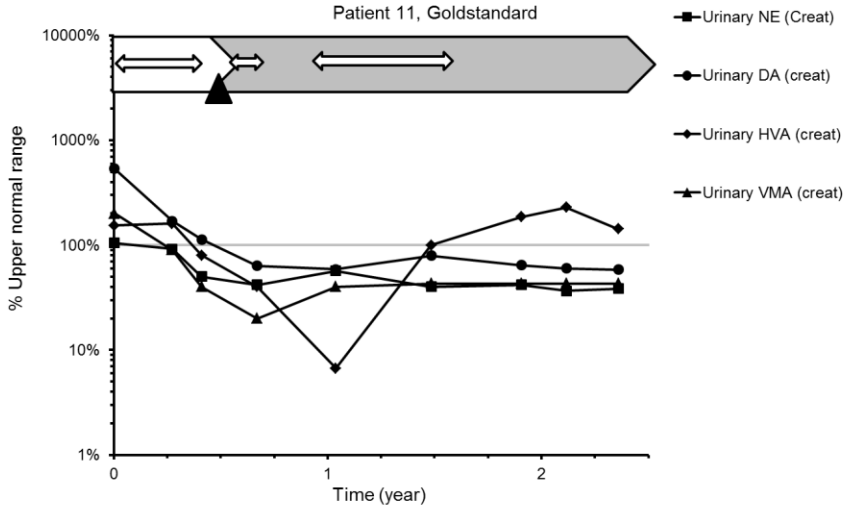


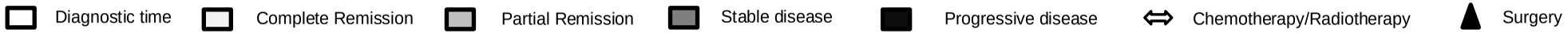
Diagnostic time | Complete Remission | Partial Remission | Stable disease | Progressive disease | ⇔ Chemotherapy/Radiotherapy | ▲ Surgery


Patient 11 Noradrenergic line
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)
Free plasma NMN
Total plasma NMN
Urinary NE (Creat)
Urinary NMN (Creat)


Patient 11 Adrenergic line
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)
Free plasma MN
Total plasma MN
Urinary E (Creat)
Urinary MN (Creat)


Patient 11 Dopaminergic line
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)
Free plasma MT
Total plasma MT
Urinary DA (Creat)
Urinary MT (Creat)
Urinary HVA (creat)


Patient 11, Goldstandard
% Upper normal range
10000%
1000%
100%
10%
1%
0
1
2
Time (year)
Urinary NE (Creat)
Urinary DA (creat)
Urinary HVA (creat)
Urinary VMA (creat)


Diagnostic time
Complete Remission
Partial Remission
Stable disease
Progressive disease
Chemotherapy/Radiotherapy
Surgery