AD_____

Award Number: DAMD17-03-1-0297

TITLE: Genomic and Expression Profiling of Benign and Malignant Nerve Sheath Tumors in Neurofibromatosis Patients

PRINCIPAL INVESTIGATOR: Matt van de Rijn, M.D., Ph.D.
Torsten Nielsen, M.D., Ph.D.
Brian Rubin, M.D., Ph.D.

CONTRACTING ORGANIZATION: Leland Stanford Junior University
Stanford, California 94305-4125

REPORT DATE: May 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2004	Annual (1 May 03-30 Apr 04)

4. TITLE AND SUBTITLE
Genomic and Expression Profiling of Benign and Malignant Nerve Sheath Tumors in Neurofibromatosis Patients

5. FUNDING NUMBERS
DAMD17-03-1-0297

6. AUTHOR(S)
Matt van de Rijn, M.D., Ph.D.
Torsten Nielsen, M.D., Ph.D.
Brian Rubin, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Leland Stanford Junior University
Stanford, California 94305-4125

E-Mail: mrijn@stanford.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates. All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The goal of this project is to identify genes involved in the malignant transformation of neurofibromas to malignant peripheral nerve sheath tumors using expression profiling and array-based comparative genomic hybridization. The significance of the genes will be validated on much larger numbers of cases using antibodies and in situ hybridization probes on tissue microarrays (TMAs). Genes will be further studied in in vitro experiments using cell lines from nerve sheath tumors. While the grant starting date was on May 1, 2003 authorization to work with human subjects was not obtained until April 1, 2004. Therefore this "annual report" will only describe the actual work performed in April 2004. Nevertheless the following progress has been made in the past year: 1. The number of cases of nerve sheath tumors available will be more than sufficient to perform the first aim of this study. 2. We have started to run expression profiling and gene microarrays on a number of nerve sheath tumors and since April 1, 2004 have analyzed six malignant peripheral nerve sheath tumors and five schwannomas. 3. We have gained much experience with in situ hybridization on TMAs. This experience will enormously benefit this project.

14. SUBJECT TERMS
Tumor development/progression, gene microarrays, expression profiling, array-based comparative genomic hybridization, tissue microarrays, immunohistochemistry

15. NUMBER OF PAGES
66

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18

Table of Contents

Award Number: DAMD17-03-1-0297

TITLE: Genomic and Expression Profiling of Benign & Malignant Nerve Sheath Tumors in Neurofibromatosis Patients

INTRODUCTION

Malignant transformation of benign neurofibromas is a life threatening complication in patients with neurofibromatosis. The goal of this study is to identify markers that may help the clinician determine whether a malignant transformation of a benign neurofibroma is actually in progress in a patient. We will accomplish this through a large scale genome-wide expression profiling study on benign lesions and malignant lesions originating in the nerve sheath to find genes that can function as markers of this malignant transformation. In this genome-wide search we will also use comparative genomic hybridization. In this technique, the presence of gene amplifications or deletions in tumor samples is determined. Subsequently we will validate and extend these findings on tissue microarrays (TMAs)containing very large numbers of these tumors using paraffin-embedded, formalin-fixed material. These TMAs will be examined by immuno-histochemistry with existing or de novo generated antisera or by in situ hybridization for the genes of interest.

The ultimate goal is to find markers that will help us distinguish benign from malignant nerve sheath tumors and that ultimately may lead to a serological marker to follow disease progression as well. In addition it can be expected that we may find several novel potential therapeutic targets for the treatment of malignant nerve sheath tumors.

BODY

The initiation of this project was delayed by eleven months by issues regarding the authorization to work with human subject material. However, it should be stressed that once I got into direct contact with Dr. Inese Beitins, the process actually went very quickly. I wish that I had known of her existence much earlier. On February 26 I sent her a letter after having been advised by her through a telephone conversation. Subsequently we had the approval to proceed by April 1. I mention this in detail because I can imagine that these issues might occur with other grants as well and I believe that an unnecessary delay of

ten months could have been prevented had I known of Dr. Beitins'existence so that I could have contacted her sooner.

Despite the delay incurred we have been able to make significant progress. In the past year I have inventoried the number of nerve sheath tumors currently available to me and have determined that these are sufficient to perform the initial phase of the study using gene microarrays to perform expression profiling and comparative genomic hybridization. As of April 1st, 2004, we also have started to perform these studies as described in the section below. Importantly, in parallel projects performed in my laboratory we have developed an enormous amount of experience with in situ hybridization on tissue microarrays and the experience gained in this area will be extremely valuable once we identify genes of interest in the nerve sheath tumor project.

Specific aim 1: "Genome-wide search for genes in nerve sheath tumors"

Initial expression profiling experiments using 40,000 elements cDNA microarrays were performed on six malignant peripheral nerve sheath tumors, five schwannomas and six synovial sarcomas (Figure 1). Gene filtering was then performed to remove genes that were poorly measured and to remove those genes that show no significant variation across the samples. Specifically, using selection criteria for signal over-background measurement of quality of the signal intensity 34,000 genes passed the filters. The subsequent filtering that selected for genes that varied at least fourfold in at least two of the arrays removed approximately 30,000 genes. In the final filtering step we removed genes that were not well measured in at least 80% of the data. As a result of these rather stringent filtering criteria, 1,920 genes were selected for the hierarchical clustering. The gene selection as described above is just one of many gene selections that we will perform on this data set and that we will continue to perform as the actual data set continues to grow through additions of more nerve sheath tumor specimens and other sarcomas. Unsupervised hierarchical clustering was performed on the 1,920 gene data set and as shown in Figure 1 all synovial sarcomas clustered on a branch distant from the other tumors. Likewise all schwannomas clustered on a separate branch. The malignant peripheral nerve sheath tumors showed a distribution over three branches, one of which (branch two) contained a majority (four cases) of the samples while the other two cases (STT3990 and STT3994) were on separate branches of the branch that contained that contained all schwannomas. These

findings show that malignant peripheral nerve sheath tumors are a heterogeneous group of tumors and they emphasize the need for detailed comparison with histologic findings and gene array studies. In the collaboration between myself and Drs. John Goldblum, Brian Rubin and Torsten Nielsen we have sufficient surgical pathology expertise to guarantee this.

Of course this data set is still quite small and much smaller than the one that we hope to obtain in the next 1 to 1 1/2 years. Nevertheless as an example of this study we have performed a SAM analysis to determine the genes that are most significant in the separation of malignant peripheral nerve sheath tumors from the other lesions. An example of this SAM analysis is shown in Table 1 where the 112 most significant genes that determined separation from MPNST from the other tumors are shown. Interestingly for several of these genes including CTHRC1, EGFR, we have already generated in situ hybridization probes in parallel projects on fibromatosis studies. These probes will act as validation tools of gene array data using tissue microarrays.

Specific aim 2: "Validation of candidate genes"
An important development in my laboratory is that we have become very familiar with the generation and use of in situ hybridization probes on our TMAs. In situ hybridization on formalin fixed, paraffin embedded material has long been problematic. We have started using a method (published by others) that incorporates tyramide amplification of signal to perform a non-radioactive in situ hybridization on our TMAs. In the appendix I have included a preprint of a paper, currently *in press* in the American Journal of Pathology, where we describe a novel marker in GIST tumors and where examples of this technique can be seen. I expect that incorporation of this technique in the nerve sheath tumor project will markedly increase the number of candidate genes that we can examine on TMAs. This is because antiserum production (as proposed in the original grant application) is a very long-term proposal with a turnaround of at least 4-6 months. In contrast, in situ hybridization probes can be generated within 10 days. We still expect to generate significant numbers of conventional antisera as they will allow us to study the proteins rather than the mRNA for interesting genes but we also expect that in situ hybridization will be used as a first determination for the significance of genes identified by expression profiling or comparative genomic hybridization.

KEY RESEARCH ACCOMPLISHMENTS

1 Inventory of nerve sheath tumors available at Stanford.
This has been completed and the number of cases will be sufficient to perform the initial phase of the study.

2 Initiation of gene array studies.
See section above.

3 Development of in situ hybridization.
We have generated a lot of experience with non-radioactive in situ bybridization on TMAs. To date we have generated 52 probes. This will tremendously speed up our ability to examine large numbers of genes on TMAs.

REPORTABLE OUTCOMES

No reportable outcomes are available. The study has really only has been started for the last month.

CONCLUSIONS

The data shown above forms an important proof of principle that we can perform these high volume experiments on the available samples. The RNA quality was excellent from the material we have collected and we expect to be able to analyze many more malignant peripheral nerve sheath tumors in the next year. Our experience with in situ hybridization will allow us to look at larger numbers of genes in more detail with a faster turnaround.

REFERENCES:

None.

APPENDICES

Figure 1.
Table 1.
Preprint The novel marker, DOG1, is expressed ubiquitously in GI stromal tumors irrespective of KIT or PDGFRA mutation status. *American Journal of Pathology, in press.*
Curriculum vitae Matt van de Rijn.

Figure 1 - page one of two

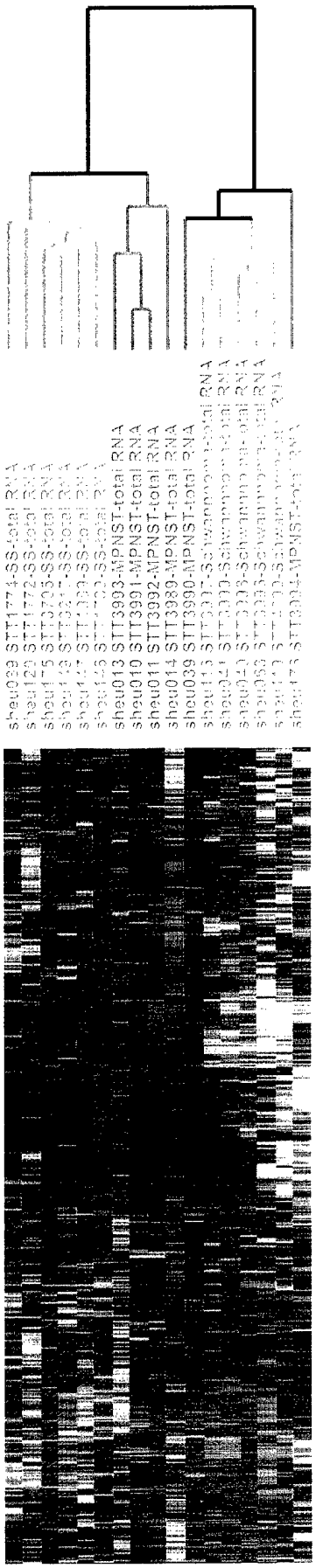


Figure 1 DAMD-17-03-1-0297

Legend to Figure 1

Unsupervised hierarchical clustering of gene expression profiling data performed on six synovial sarcomas, six malignant peripheral nerve sheath tumors and five schwannomas. All samples were analyzed on the same print run of 40,000 element cDNA gene microarrays. Data were entered in the Stanford microarray database and gene filtering was performed as described in the text. Subsequently the data were grouped together using the Cluster program developed by Mike Eisen that tumors with similar gene expression profiles across the genes selected were grouped together and genes that showed similar expression profiles across the 17 specimens tested were grouped together. The results were depicted in a "heat map" where the intersection between a gene (in rows) and a tumor specimen (in columns) was labeled red when the gene was relatively highly expressed in that sample compared to the other samples. The intersection was labeled green when the expression was relatively low. Using this unsupervised method of analysis it is clear that all synovial sarcomas (in blue) clustered together on one branch while all schwannomas (in green) likewise cluster on a separate branch. The majority of the MPNSTs tested so far (four cases) cluster on a branch separate from all other cases while the remaining two MPNST cases cluster on separate branches of the schwannoma group. By inspecting the heat map it can be seen that, as expected, the malignant peripheral nerve sheath tumors and the synovial sarcomas share expression in a large number of genes. However there is a significant number of genes that are unique to the MPNSTs and it will be these genes that will be initially studied in more detail if they can be verified in gene microarray experiments larger numbers of MPNSTs. The SAM analysis (Table 1) highlights 112 genes that are significant in distinguishing the 6 MPNSTs from the other specimens.

TABLE 1

Significant Genes List

Input Parameters

Imputation Engine	**10-Nearest Neighbor Imputer**
Data Type	**Two Class, unpaired data**
Data in log scale?	**TRUE**
Number of Permutations	**1000**
Blocked Permutation?	**FALSE**
RNG Seed	**1234567**
(Delta, Fold Change)	**(0.40799,)**
(Upper Cutoff, Lower Cutoff)	**(1.09743, -∞)**

Computed Quantities

Computed Exchangeability Factor S0	**0.945709644**
S0 percentile	**0.82**
False Significant Number (Median, 90 percentile)	**(7.08750, 29.92500)**
False Discovery Rate (Median, 90 percentile)	**(6.32812, 26.71875)**
PI0Hat	**0.7875**

112 Positive Significant Genes

Row	Gene Name	Gene ID	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value (%)
439	112583 || POSTN || periostin, osteoblast specific factor || Hs.136348	1	2.498965525	4.562909091	1.825919183	9.47673	1.96875
411	112249 || LRRC17 || leucine rich repeat containing 17 || Hs.288720	1	2.237658482	3.650363636	1.631331888	16.83591	1.96875
441	220604 || CTHRC1 || collagen triple helix repeat containing 1 || Hs.283713	1	2.144621646	3.422257576	1.595739548	8.73993	1.96875
440	308692 || POSTN || periostin, osteoblast specific factor || Hs.136348	1	2.131218847	3.559030303	1.669950652	8.70109	1.96875
413	310753 || LRRC17 || leucine rich repeat containing 17 || Hs.288720	1	2.127896524	3.447015152	1.619916717	13.42531	1.96875
369	99553 || RGS4 || regulator of G-protein signalling 4 || Hs.386726	1	2.07153401	3.819727273	1.843912412	8.10503	1.96875
196	104616 || PTN || pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1) || Hs.44	1	2.03030837	3.179242424	1.565891404	7.77272	1.96875
352	99425 || || Transcribed sequences || Hs.529878	1	1.906883192	3.398545455	1.782251513	8.73941	1.96875
595	114642 || EGFR || epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) || Hs.77432	1	1.811998976	3.373045455	1.861505166	8.09617	1.96875
1326	117895 || HFL1 || H factor (complement)-like 1 || Hs.296941	1	1.801222467	3.428333333	1.903336981	5.55673	1.96875
194	106895 || C17 || cytokine-like protein C17 || Hs.13872	1	1.793134532	2.800863636	1.561993027	7.64537	1.96875
211	119739 || KIAA2028 || similar to PH (pleckstrin homology) domain || Hs.255938	1	1.772994628	3.801909091	2.144343266	6.26947	1.96875
412	110424 || XRCC1 || X-ray repair complementing defective repair in Chinese hamster cells 1 || Hs.98493	1	1.730029111	2.643560606	1.528044002	8.34350	1.96875
402	248886 || || ||	1	1.723287628	2.612772727	1.516155913	4.88238	1.96875
1329	107265 || COL12A1 || collagen, type XII, alpha 1 || Hs.101302	1	1.719152154	2.741515152	1.594690234	6.10305	1.96875
401	224472 || || Transcribed sequence with strong similarity to protein pdb:1BGM (E. coli) O Chain O, Beta-Galactosidase || Hs.387246	1	1.678334362	3.064227273	1.825754952	4.06973	1.96875
206	119676 || || MRNA full length insert cDNA clone EUROIMAGE 994183 || Hs.71947	1	1.651920855	2.532333333	1.532962869	5.40119	1.96875
347	222205 || SEMA3A || sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A || Hs.252451	1	1.645727371	2.917272727	1.772634264	5.03821	1.96875
1735	115674 || TM4SF9 || transmembrane 4 superfamily member 9 || Hs.8037	1	1.636089944	2.910636364	1.779019775	4.53157	1.96875
1742	99502 || || ||	1	1.610966209	2.395787879	1.487174508	4.26634	1.96875
1744	106847 || NRN1 || neuritin 1 || Hs.103291	1	1.604237802	3.225530303	2.010631029	2.82984	1.96875
403	101078 || || ||	1	1.584066494	2.406257576	1.519038237	4.05597	1.96875
1330	111665 || TIEG || **TGFB inducible early growth response || Hs.82173	1	1.58137057	2.28969697	1.4479193	4.43302	1.96875
596	309699 || EGFR || epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) || Hs.77432	1	1.54841888	2.905151515	1.876205175	8.47433	1.96875
594	108730 || || CDNA: FLJ23165 fis, clone LNG09846 || Hs.279898	1	1.537913074	3.195030303	2.077510334	12.71387	1.96875
397	104835 || MYO1B || myosin IB || Hs.121576	1	1.53624003	2.426090909	1.579239482	5.23870	1.96875
1307	111660 || || MRNA full length insert cDNA clone EUROIMAGE 248114 || Hs.231971	1	1.53092024	2.650681818	1.731430383	4.72455	1.96875
678	120018 || C5orf13 || chromosome 5 open reading frame 13 || Hs.508741	1	1.527209387	2.797121212	1.831524371	3.39428	1.96875
726	108290 || NOPE || likely ortholog of mouse neighbor of Punc E11 || Hs.20924	1	1.526266455	2.944848485	1.929445855	5.26460	1.96875
351	222443 || KIAA0922 || KIAA0922 protein || Hs.511944	1	1.525653482	2.254878788	1.477975709	4.58324	1.96875
438	107998 || FAP || fibroblast activation protein, alpha || Hs.436852	1	1.486713433	2.510151515	1.688389611	4.01014	1.96875
407	311649 || ASAM || adipocyte-specific adhesion molecule || Hs.135121	1	1.477668694	3.034666667	2.05368543	4.79512	1.96875
597	99435 || EGFR || epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) || Hs.77432	1	1.473733265	2.872454545	1.949100705	7.01630	1.96875
699	109176 || || ||	1	1.440807558	3.242121212	2.250211136	2.50095	1.96875

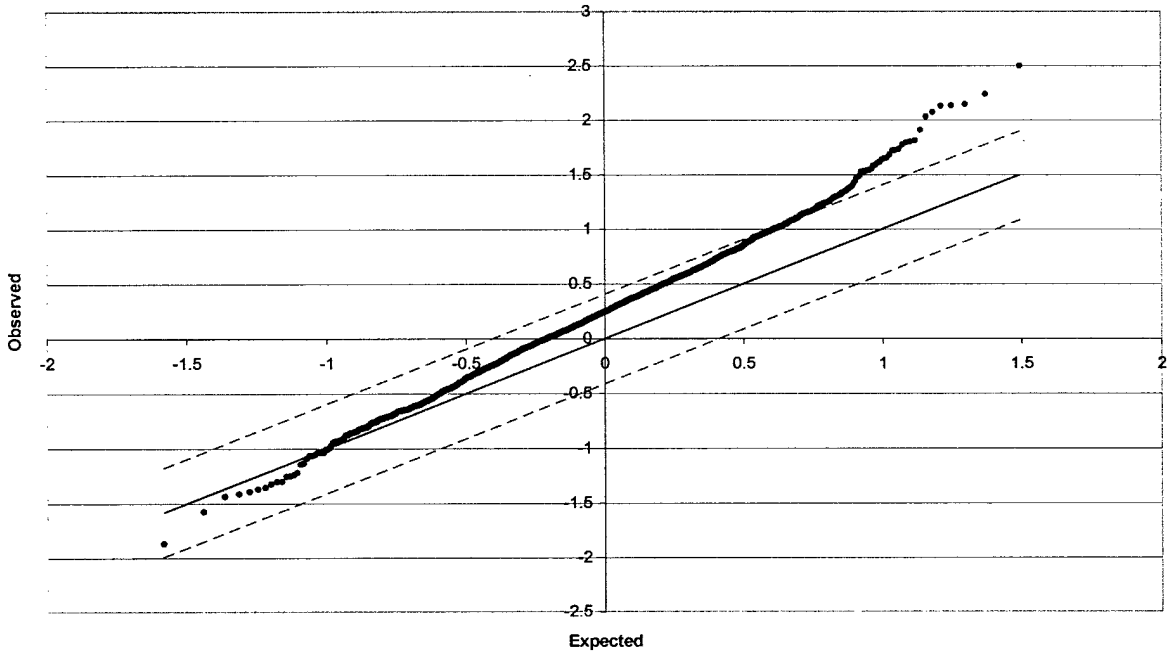
1737	117428 || TM4SF9 || transmembrane 4 superfamily member 9 || Hs.8037	1	1.420551688	2.709212121	1.907154906	8.31670	1.96875
247	106800 || SYTL4 || synaptotagmin-like 4 (granuphilin-a) || Hs.376981	1	1.39484643	2.39369697	1.716100725	3.14201	1.96875
1325	118248 || HFL1 || H factor (complement)-like 1 || Hs.296941	1	1.385715426	2.530166667	1.825891968	3.42071	1.96875
357	311130 || HAS2 || hyaluronan synthase 2 || Hs.159226	1	1.381114398	2.463939394	1.784022668	11.94781	1.96875
731	221983 || KLHL8 || kelch-like 8 (Drosophila) || Hs.372101	1	1.3681675	1.904515152	1.392018997	3.85484	1.96875
1290	113985 || || Clone IMAGE:5278074, mRNA || Hs.26409	1	1.366200107	2.675772727	1.958551104	2.09850	1.96875
1327	307158 || HF1 || H factor 1 (complement) || Hs.278568	1	1.351882387	2.524242424	1.867205645	3.45008	2.91666667
345	105845 || COL1A2 || collagen, type I, alpha 2 || Hs.232115	1	1.344539149	2.079151515	1.546367405	4.21749	2.91666667
610	107468 || STEAP || six transmembrane epithelial antigen of the prostate || Hs.61635	1	1.341171642	2.561909091	1.910202252	3.48179	2.91666667
408	116073 || ASAM || adipocyte-specific adhesion molecule || Hs.135121	1	1.334969315	2.287909091	1.713828973	4.01309	2.91666667
250	108540 || MLSTD1 || male sterility domain containing 1 || Hs.134497	1	1.333711478	2.02119697	1.515467928	4.47635	2.91666667
579	116829 || IGF2 || insulin-like growth factor 2 (somatomedin A) || Hs.349109	1	1.316475972	2.326136364	1.766941755	3.53605	2.91666667
346	106595 || DKFZp686A17109 || hypothetical protein DKFZp686A17109 || Hs.369523	1	1.315124404	2.010787879	1.528971611	4.48920	2.91666667
1739	102761 || EDG2 || endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2 || Hs.75794	1	1.310611227	2.953333333	2.253401521	2.23197	2.91666667
1319	118928 || TIMP1 || tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) || Hs.446641	1	1.307041008	1.993515152	1.525212399	3.42281	2.91666667
1280	118070 || DKK2 || dickkopf homolog 2 (Xenopus laevis) || Hs.211869	1	1.298317162	2.109787879	1.625017322	5.77322	2.91666667
445	103381 || PLOD2 || procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2 || Hs.41270	1	1.294390952	1.775424242	1.371629058	3.69645	2.91666667
417	223884 || PRRX1 || paired related homeobox 1 || Hs.443452	1	1.292280155	2.605757576	2.016402996	3.32533	2.91666667
1738	226503 || C1orf24 || chromosome 1 open reading frame 24 || Hs.48778	1	1.291536368	2.404848485	1.862006014	3.21995	2.91666667
199	101968 || SPRY2 || sprouty homolog 2 (Drosophila) || Hs.18676	1	1.289037991	2.20030303	1.706934198	4.46361	2.91666667
443	116402 || || MRNA; cDNA DKFZp686G03142 (from clone DKFZp686G03142) || Hs.289044	1	1.275587569	1.74030303	1.364314824	3.41619	4.00423729
361	100151 || LIFR || leukemia inhibitory factor receptor || Hs.446501	1	1.274856868	1.899560606	1.490018726	3.63359	4.00423729
187	110597 || NEF3 || neurofilament 3 (150kDa medium) || Hs.458657	1	1.265827763	2.375030303	1.876266561	4.48930	4.00423729
727	115261 || ENAH || enabled homolog (Drosophila) || Hs.446693	1	1.254547395	2.363212121	1.883716893	3.09159	4.00423729
373	317940 || ITGA4 || **integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor) || Hs.528404	1	1.253590071	2.298121212	1.833231823	3.14574	4.00423729
653	319669 || || CDNA clone MGC:52263 IMAGE:4123447, complete cds || Hs.251664	1	1.248032848	2.540909091	2.035931262	2.99297	4.25675676
91	118015 || PEG10 || paternally expressed 10 || Hs.137476	1	1.244945006	2.407530303	1.9338447	3.28927	4.25675676
410	111639 || || ||	1	1.243928797	1.876060606	1.508173628	4.07671	4.25675676
424	119326 || RAB23 || RAB23, member RAS oncogene family || Hs.94769	1	1.236085487	1.771484848	1.433141046	3.25811	4.25675676
212	100732 || ECM2 || extracellular matrix protein 2, female organ and adipocyte specific || Hs.117060	1	1.236081775	2.134242424	1.726619119	4.03006	4.25675676
598	101379 || || ||	1	1.233924901	2.226121212	1.804097811	3.79646	4.25675676
197	100071 || || Transcribed sequences || Hs.32135	1	1.233257794	2.349909091	1.9054484	3.67514	4.25675676
634	101410 || IQGAP3 || IQ motif containing GTPase activating protein 3 || Hs.133294	1	1.229481227	2.220833333	1.806317401	3.48407	4.25675676
367	311177 || LOX || lysyl oxidase || Hs.102267	1	1.225014059	1.744090909	1.423731341	2.98819	4.25675676
205	104532 || STXBP6 || syntaxin binding protein 6 (amisyn) || Hs.99291	1	1.222217886	1.826272727	1.494228442	3.93081	4.25675676
372	106873 || || Human S6 H-8 mRNA expressed in chromosome 6-suppressed melanoma cells. || Hs.446408	1	1.222129016	2.322424242	1.900310207	2.50462	4.25675676
435	106250 || TMEM2 || transmembrane protein 2 || Hs.160417	1	1.217934553	2.109393939	1.731943587	3.40537	4.25675676
1328	119752 || DKFZP586L151 || DKFZP586L151 protein || Hs.43658	1	1.213684925	2.070878788	1.706273798	3.18066	4.25675676
1023	110878 || DOCK11 || dedicator of cytokinesis 11 || Hs.107513	1	1.207685873	1.81969697	1.506763481	2.85151	4.25675676
515	105460 || WDTC1 || **WD and tetratricopeptide repeats 1 || Hs.172825	1	1.207015281	1.766212121	1.463288948	2.66131	4.25675676
404	330898 || || Similar to pyrroline-5-carboxylate reductase 1 isoform 2; P5C reductase (LOC400634), mRNA || Hs.512314	1	1.202432032	1.869060606	1.554400212	3.43121	5.22321429
454	105760 || DDEF1 || development and differentiation enhancing factor 1 || Hs.386779	1	1.19572801	2.137878788	1.787930675	4.30948	5.22321429
358	115196 || LOX || lysyl oxidase || Hs.102267	1	1.187400027	1.74619697	1.470605465	2.88180	5.22321429
437	107150 || || CDNA FLJ35517 fis, clone SPLEN2000698 || Hs.519270	1	1.185192406	1.731515152	1.460957009	3.33179	5.22321429
427	108628 || ADAM12 || a disintegrin and metalloproteinase domain 12 (meltrin alpha) || Hs.8850	1	1.178616151	1.968181818	1.669909085	2.94814	5.22321429
516	106445 || MGC9850 || hypothetical protein MGC9850 || Hs.222061	1	1.173921935	2.153939394	1.834823364	2.79660	5.22321429
677	111520 || || MRNA; cDNA DKFZp586L0120 (from clone DKFZp586L0120) || Hs.98314	1	1.173326924	2.347454545	2.000682417	2.36489	5.22321429
405	110180 || PCOLCE || procollagen C-endopeptidase enhancer || Hs.202097	1	1.172412625	1.777424242	1.516039835	2.41316	5.22321429
1256	100028 || CLECSF2 || **C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced) || Hs.85201	1	1.166683601	1.825181818	1.564418851	2.99191	5.22321429
616	99493 || CDCA1 || cell division cycle associated 1 || Hs.234545	1	1.160548388	1.889924242	1.628475178	3.30904	5.22321429
409	311729 || CXCL6 || chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2) || Hs.164021	1	1.157630094	1.605909091	1.387238548	3.15881	5.22321429
651	116998 || CCDC8 || coiled-coil domain containing 8 || Hs.97876	1	1.156217738	1.715106061	1.48337636	3.24975	5.22321429
425	101543 || ADAM12 || a disintegrin and metalloproteinase domain 12 (meltrin alpha) || Hs.8850	1	1.155618781	2.379242424	2.058847142	1.82285	5.22321429
360	106544 || SHMT2 || **serine hydroxymethyltransferase 2 (mitochondrial) || Hs.75069	1	1.154976376	1.87069697	1.619684184	2.95897	5.22321429
257	104207 || FZD6 || frizzled homolog 6 (Drosophila) || Hs.114218	1	1.154810442	1.996212121	1.728605881	3.17579	5.22321429
350	103810 || PDE8B || phosphodiesterase 8B || Hs.78106	1	1.151726636	1.963166667	1.704542211	3.02629	5.22321429
213	98915 || OGN || osteoglycin (osteoinductive factor, mimecan) || Hs.109439	1	1.150375978	2.302545455	2.001559054	6.90794	5.22321429
1736	312147 || TM4SF9 || transmembrane 4 superfamily member 9 || Hs.8037	1	1.149928511	2.100151515	1.826332242	4.57315	5.22321429

387	100497 || MBNL1 || muscleblind-like (Drosophila) || Hs.28578	1	1.147032892	1.945757576	1.696339825	9.26944	5.22321429
1331	309530 || CXCL14 || chemokine (C-X-C motif) ligand 14 || Hs.24395	1	1.146247354	2.257090909	1.969113299	3.94158	5.22321429
202	310309 || ENPP2 || ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin) || Hs.23719	1	1.145241645	1.933545455	1.688329675	4.20937	5.22321429
782	114093 || FZD3 || frizzled homolog 3 (Drosophila) || Hs.40735	1	1.141748823	1.784833333	1.563245171	2.00932	5.22321429
1148	319580 || FST || follistatin || Hs.9914	1	1.141640136	1.896333333	1.661060498	4.73318	5.22321429
447	187712 || STK38L || serine/threonine kinase 38 like || Hs.184523	1	1.141562622	1.868727273	1.636990593	4.88712	5.22321429
392	117540 || || Transcribed sequences || Hs.128809	1	1.136857639	1.754409091	1.543209133	2.79309	5.30048077
998	109251 || RRM2 || ribonucleotide reductase M2 polypeptide || Hs.226390	1	1.131628031	2.198772727	1.943017199	3.18905	5.30048077
249	330561 || PRKCQ || protein kinase C, theta || Hs.408049	1	1.12658191	2.063	1.831202846	17.03237	5.30048077
370	100008 || IGSF10 || immunoglobulin superfamily, member 10 || Hs.386296	1	1.126401978	1.818287879	1.614244217	3.15050	5.30048077
687	111691 || EDNRA || endothelin receptor type A || Hs.211202	1	1.125991005	1.913545455	1.699432275	4.28225	5.30048077
1324	109102 || MGP || matrix Gla protein || Hs.365706	1	1.122712847	2.11830303	1.886771881	2.37488	5.30048077
1026	103897 || RHOBTB3 || Rho-related BTB domain containing 3 || Hs.31653	1	1.113778714	1.734212121	1.55705267	3.26273	6.328125
385	330992 || G3BP2 || Ras-GTPase activating protein SH3 domain-binding protein 2 || Hs.303676	1	1.106040355	1.625575758	1.469725539	3.84376	6.328125
512	307378 || STC1 || stanniocalcin 1 || Hs.25590	1	1.104105893	1.783257576	1.615114626	3.87858	6.328125
499	314788 || || CDNA FLJ41501 fis, clone BRTHA2006975 || Hs.389638	1	1.103899985	1.726939394	1.564398422	2.22770	6.328125
446	99834 || || ||	1	1.099215031	1.570787879	1.429008732	3.39925	6.328125
693	100664 || SVIL || supervillin || Hs.163111	1	1.098954578	1.916121212	1.743585449	2.16294	6.328125
428	226520 || ADAM12 || a disintegrin and metalloproteinase domain 12 (meltrin alpha) || Hs.8850	1	1.098456828	1.794969697	1.634083062	3.07196	6.328125
434	102456 || PTGIS || prostaglandin I2 (prostacyclin) synthase || Hs.302085	1	1.09742759	1.695015152	1.544534844	2.44386	6.328125

Significant: 112
Median # false significant: 7.08750

SAM Plot

Delta 0.40799
Fold Change



The novel marker, *DOG1*, is expressed ubiquitously in GI Stromal Tumors irrespective of *KIT* or *PDGFRA* mutation status

Robert B. West[1], Christopher L. Corless[2], Xin Chen[7], Brian P. Rubin[3], Subbaya Subramanian[1], Kelli Montgomery[1], Shirley Zhu[1], Catherine A. Ball[4], Torsten O. Nielsen[5], Rajiv Patel[6], John R. Goldblum[6], Patrick O. Brown[7], Michael C. Heinrich[8], Matt van de Rijn[1].

Departments of [1]Pathology, [4]Genetics, and [7]Biochemistry and Howard Hughes Medical Institute, Stanford University Medical Center, Departments of [2]Pathology and [8]Medicine, Oregon Health & Science University Cancer Institute and Portland VA Medical Center, Portland, OR, [3]Department of Anatomical Pathology, University of Washington Medical Center, Seattle, WA, [5]Department of Pathology and Genetic Pathology Evaluation Centre, Vancouver General Hospital, Vancouver BC, [6]Dept of Anatomic Pathology, Cleveland Clinic Foundation, Cleveland, OH.

Address correspondence to:

Matt van de Rijn: mrijn@stanford.edu

Department of Pathology, Stanford University Medical Center

300 Pasteur Drive, Stanford, CA, 94305

Phone: 650-498-7154

Abstract:

We recently characterized gene expression patterns in GISTs using cDNA microarrays, and found that the gene *FLJ10261* (DOG1, Discovered on GIST-1), encoding a hypothetical protein, was specifically expressed in GISTs. The immunoreactivity of a rabbit antiserum to synthetic DOG1 peptides was assessed on two soft tissue tumor microarrays (TMAs). The TMAs included 587 soft tissue tumors, with 149 GISTs, including 127 GIST cases for which the *KIT* and *PDGFRA* mutation status was known. Immunoreactivity for DOG1 was found in 136 of 139 (97.8%) of scorable GISTs. All 7 GIST cases with a *PDGFRA* mutation were DOG1 positive, while most of these failed to react for KIT. The immunohistochemical findings were confirmed with in situ hybridization probes for *DOG1*, *KIT* and *PDGFRA*. Other neoplasms in the differential diagnosis of GIST, including desmoid fibromatosis (0/17) and Schwannoma (0/3), were immunonegative for DOG1. Only 4 out of 438 non-GIST cases were immunoreactive for DOG1.

DOG1, a protein of unknown function, is expressed strongly on the cell surface of GISTs and is rarely expressed in other soft tissue tumors. Reactivity for DOG1 may aid in the diagnosis of GISTs, including *PDGFRA* mutants that fail to express KIT antigen, and lead to appropriate treatment with imatinib mesylate, an inhibitor of the KIT tyrosine kinase.

Introduction:

Gastrointestinal stromal tumors occur in the wall of the bowel and have been proposed to arise from the interstitial cells of Cajal. The differential diagnosis of these tumors includes desmoid fibromatosis, Schwannoma, leiomyosarcoma, and, in some cases, high grade sarcomas [1]. Accurate diagnosis of GIST is important, because imatinib mesylate has been shown to significantly inhibit these tumors presumably through inhibition of the KIT tyrosine kinase receptor, which is highly expressed in these tumors [2-5]. As a result, the diagnosis of GIST relies heavily on KIT immunoreactivity. Current recommendations in the literature emphasize a diffuse, strong KIT immunoreactivity for the diagnosis of GIST [6]. CD34 immunostaining can also aid in the diagnosis, but a subset of cases is immunonegative while many other types of sarcomas are immunoreactive for this marker [7-10]. In the vast majority of GISTs, high levels of KIT expression are accompanied by a *KIT* gene mutation in exon 9, 11, 13 or 17 [11,12].

Recently, a subset of GISTs have been found to have *PDGFRA* mutations rather than *KIT* mutations [13,14]. Patients with GISTs containing mutations in *PDGFRA* may still benefit from imatinib therapy, but these tumors often fail to react with antibodies against KIT and hence may remain undiagnosed as GIST [2]. In addition, some GISTs with KIT mutations may have low KIT expression by immunohistochemistry yet will still respond to imatinib therapy [15].

Although much work has been done on the biology of GISTs and KIT, additional insight has recently been gained through gene microarray studies [16-18]. These studies have identified a number of genes whose expression is relatively increased compared to other soft tissue tumors. This includes genes known to be involved with GISTs, such as

KIT and *CD34,* but also includes a number of genes that have not been well characterized. We have generated an antiserum against one GIST specific gene, encoding for the hypothetical protein FLJ10261, which we have named "Discovered on GIST 1" (DOG1). Using immunohistochemistry with this antiserum and in situ hybridization with *DOG1*-specific probes, we show that DOG1 is highly expressed not only in typical GISTs but also in *KIT*-mutation negative GIST.

Materials and Methods:

Tissue Microarray

The studies described here were performed with the approval of the Institutional Review Board at Stanford University Hospital. Two TMAs were used for this study. The first TMA contained 460 different soft tissue tumors from 421 patients, with each tumor represented by two cores. The samples were distributed over two array blocks that were constructed using a technique previously described [19] with a tissue arrayer from Beecher Instruments, Silver Spring, MD. 0.6 mm cores were taken from paraffin embedded soft tissue tumors archived from the Stanford University Medical Center between 1995 and 2001. This array has also been used for characterization of Apolipoprotein D expression [20]. The second TMA used GISTs that were obtained from the pathology archives of Oregon Health and Science University Hospital, the Portland VA Medical Center and the Kaiser Permanente Northwest Regional Laboratory. This single-block array consisted of 0.6 mm cores from formalin-fixed, paraffin-embedded tumor assembled using a semi-automated tissue arrayer [21]. There was one core for each tumor, and all of the GISTs on this TMA were analyzed for mutations in exons 9, 11, 13 and 17 of the *KIT* gene using a combination of denaturing HPLC and direct sequencing, as previously described [13,22]. *KIT* wild-type tumors included on the array were also screened for mutations in exons 12 and 18 of the *PDGFRA* gene [13].

Antibody Generation

The cDNA-derived protein sequence of *DOG1* showed no significant homology with other genes, including the *KIT* gene. A rabbit polyclonal antibody was raised by

injecting 3 peptides derived from the gene sequence (Applied Genomics Inc. (AGI), Hunstville, AL). These peptides have no sequence homology to KIT. The peptides were synthesized by standard FMOC chemistry: Peptide 1 EEAVKDHPRAEYEARVLEKSLK; Peptide 2 DHEECVKRKQRYEVDYNLE; Peptide 3 KEKVLMVELFMREEQDK . The peptides were conjugated to KLH and injected into two out-bred rabbits. The serum (S284) was harvested after the rabbits demonstrated a significant anti-peptide titer. Affinity-purified antibodies were obtained by passing the antiserum over an affinity column conjugated with the three peptides; bound antibodies were eluted with a pH gradient.

Immunohistochemistry

Primary antibodies were directed towards DOG1 (S284, AGI, Rabbit polyclonal, 1:50) and KIT (DAKO, Carpinteria, CA Rabbit polyclonal, 1:50). Serial sections of 4 μM were cut from the tissue array blocks, deparaffinized in xylene, and hydrated in a graded series of alcohol. Staining was then performed using the EnVision+ anti-rabbit system (DAKO).

In situ hybridization

In situ hybridization of TMA sections was performed based on a protocol published previously [23,24]. Briefly, digoxigenin (DIG)-labeled sense and anti-sense RNA probes are generated by PCR amplification of 400 to 600 bp products with the T7 promoter incorporated into the primers. In vitro transcription was performed with a DIG RNA-labeling kit and T7 polymerase according to the manufacturer's protocol (Roche

Diagnostics, Indianapolis, IN). 5um thick sections cut from the paraffin blocks, deparaffinized in xylene, were hydrated in graded concentrations of ethanol for 5 minutes each. Sections were then incubated with 1% hydrogen peroxide, followed by digestion in 10ug/ml of proteinase K at 37°C for 30 minutes. Sections were hybridized overnight at 55°C with either sense or antisense riboprobes at 200ng/ml dilution in mRNA hybridization buffer (Dako). The following day, sections were washed in 2xSSC and incubated with 1:35 dilution of RNase A cocktail (Ambion, Austin, TX) in 2xSSC for 30minutes at 37°C. Next, sections were stringently washed in 2X SSC/50% formamide twice, followed by one wash at 0.08X SSC at 50 °C. Biotin blocking reagents (Dako) were applied to the section to block the endogenous biotin. For signal amplification, a HRP-conjugated rabbit anti-DIG antibody (Dako) was used to catalyze the deposition of biotinyl tyramide, followed by secondary streptavidin complex (GenPoint kit; Dako). The final signal was developed with DAB (GenPoint kit; Dako), and the tissues were counterstained in hematoxylin for 15 seconds.

Scoring of Immunohistochemistry and in situ hybridization

Cores were scored as follows. A score of "0" was given for absent or insignificant staining: less than 5% tumor cells with light brown staining. A score of "1" was given for unscorable cores. A score of "2" was given for light brown stain in greater than 5% of tumor cells or dark brown stain in less than 50% of tumor cells. A score of "3" was given for dark brown staining in greater that 50% tumor cells. Non-tumor cells and cells of unknown origin were not scored. The cores were independently reviewed by two

pathologists (RBW and MvdR) and disagreements were reviewed together to achieve a consensus score.

Digital image collection and data analysis

To aid in the analysis of numerous tissue cores stained by immunohistochemistry and in situ hybridization, digital images were collected using the BLISS instrument (Bacuslabs, Lombard IL; http://bacuslabs.com). Scoring results were combined using Deconvoluter and represented in Treeview [25], as shown on the accompanying website (http://microarray-pubs.stanford.edu/tma_portal/dog1/), where over 4,000 digital images are available.

Results:

Previously, we examined the gene expression profile of GISTs using cDNA microarrays and identified a number of the genes, in addition to the *KIT* gene, that demonstrated a specific pattern of elevated mRNA expression in GISTs [18]. Figure 1 shows the relative level of mRNA expression for one of these genes, *DOG1 (FLJ10261)*, compared with *KIT* in a variety of soft tissue tumors, including those in the differential diagnosis of GIST. Searches failed to show any sequence similarity between the genes on either the DNA or protein level.

A rabbit antiserum was generated against synthetic peptides derived from the putative coding sequence of DOG1. Antiserum immunoreactivity was characterized on two separate TMAs containing soft tissue tumors. The first TMA contained 460 different soft tissue tumor samples representing over 50 different diagnostic entities [20]. This array included 22 KIT-immunoreactive GISTs. The second TMA included 127 GIST cases for which the *KIT* and *PDGFRA* mutation status was previously determined. On this TMA there were 102 cases with an activating mutation in *KIT*, 8 cases with a mutation in *PDGFRA*, and 17 cases that were wild-type for both kinases but nevertheless had clinical, histologic, and immunophenotypic features typical for GIST.

In these two TMAs, 136 of 139 scorable GISTs (97.8%) demonstrated immunoreactivity with DOG1 antiserum (Figures 2 and 3, Table 1). The staining observed with DOG1 antisera appeared predominately localized to the plasma membrane (Figure 4A). In some very strongly immunoreactive samples, the subcellular distribution of the staining could not be evaluated (Figure 4B). Mast cells present in some of the samples, for example synovial sarcoma, were strongly immunoreactive as well (Figure

4C), while the same samples showed only weak staining in the mast cells with KIT antibodies. We confirmed these results with in situ hybridization studies (Figures 5 and 6). Interestingly, DOG1 antisera stained all 8 scorable *PDGFRA*-mutant GISTs (1 case from 1st TMA and 7 cases from 2nd TMA), while the KIT antibody staining was weak in 3 of these cases and negative in the remaining 5. These findings were further extended by in situ hybridization with *PDGFRA* (Figure 6). *PDGFRA* expression was predominately, but not exclusively, present in the *PDGFRA*-mutant GISTs. 5 of 6 (83%) scorable *PDGFRA*-mutant GISTs were positive for *PDGFRA* ISH (Figure 2 and 3, table 1). In contrast, only 10 of 70 (14%) *KIT*-mutant and KIT-wildtype GISTs were positive for *PDGFRA* ISH. Correlation of *KIT* ISH with KIT immunohistochemistry was good, with the ISH signal detectable in almost all immunopositive cases (Figure 2). However, a difference was seen in the *PDGFRA*-mutant GISTs with regard to KIT expression.Three cases were immunopositive for KIT, but only one case was positive by *KIT* ISH. Hierarchical clustering analysis of IHC and ISH data was performed as previously described [25]. Among these parameters – KIT IHC, *KIT* ISH, DOG1 IHC, *DOG1* ISH, and *PDGFRA* ISH – the most distinguishing feature was *PDGFRA* ISH positivity (Figure 2), with overexpression of PDGFRA by *PDFGRA* ISH seen in only in a small subset of GISTs. Images of all cores from both TMAs were digitally captured and are available at the accompanying website (http://microarray-pubs.stanford.edu/tma_portal/dog1/).

From the 460 tumor samples that were not classified as GIST in the first TMA, only four cases that were not histologically and immunophenotypically consistent with GIST were immunoreactive with DOG1 antiserum: 1 synovial sarcoma (1/20 = 5%), 1 (1/40 = 2.5%) leiomyosarcoma, 1 (1/4 = 25%) fibrosarcoma, and (1/9 = 11%) 1 Ewing's

sarcoma/PNET. Of the 40 leiomyosarcomas, 17 originated in the abdomen and none of these were DOG1 immunoreactive. Other tumors in the GIST differential diagnosis failed to stain with the DOG1 antisera. These include desmoid fibromatosis (17 cases) and Schwannoma (3 cases). Parenthetically, under the staining conditions used, none of the fibromatosis cases were positive for KIT by immunohistochemistry or in situ hybridization. One leiomyosarcoma was positive for KIT immunohistochemistry only (TMA 3725). Interestingly, the staining was exclusively in a diffuse nuclear pattern. This tumor was negative for DOG1 by both immunohistochemistry and in situ hybridization and for *KIT* in situ hybridization.

Seven cases in the first TMA, not counted among the 22 unequivocal GISTs, showed histologic features indeterminate between GIST and smooth muscle tumor. All of these tumors were located in the wall of the stomach or intestine, with four tumors from the stomach, one from the duodenum, one from the gastro-esophageal junction, and one from the rectum. All seven cases were negative for KIT by immunohistochemistry and thus might not be considered GISTs according to current recommendations [6]. However, four of the seven cases were positive by *KIT* in situ hybridization, while DOG1 immunoreactivity was seen in two cases, and all seven cases were positive for *DOG1* by in situ hybridization. Furthermore, two cases (TMA 863 and 3696) were positive for *PDGFRA* in situ hybridization. Subsequent sequence analysis of cases 863 and 3696 revealed a point mutation and a deletion in exon 18 of *PDGFRA*, respectively. To date, such mutations have only been described in GISTs. We conclude that the seven KIT immunonegative cases with morphologic features between GIST and smooth muscle tumor actually represent GISTs.

We also stained a tissue microarray containing a spectrum of normal tissues with the DOG1 antiserum (data not shown). We observed staining in the epithelium of breast, prostate, salivary gland, liver, stomach, testis, pancreas, and gallbladder. The pattern of DOG1 immunostaining of the Interstitial Cells of Cajal was similar to KIT. In addition, DOG1 antiserum reacted with a number of tumor cores in a carcinoma array, including some that did not stain with KIT antiserum (data not shown).

Discussion:

GISTs have a high rate of local recurrence [1]. Imatinib, a small molecule inhibitor of several type III receptor tyrosine kinases, including KIT and PDGFRA, has demonstrated promise in controlling GIST growth [3-5]. The majority of GISTs (80-85%) harbor oncogenic mutations of KIT, and for this reason KIT has been regarded as the primary target for imatinib therapy. Indeed, initial trials of imatinib were limited to KIT-immunoreactive GISTs. Recently it was discovered that a subset of GISTs (5-7%) has activating mutations of PDGFRA [13,14]. Most of these tumors are weak or negative in immunostaining for KIT, which may lead to underdiagnosis and possible withholding of imatinib therapy. Furthermore, identification of *PDGFRA*-mutant GISTs requires molecular analysis, a laborious process that is not ideal for application in a routine clinical setting.

In this paper, we demonstrate that a novel gene, *DOG1*, identified in a DNA microarray analysis of gene expression patterns as associated with GIST, is highly expressed in both *KIT*- and *PDGFRA*- mutant GISTs. Expression of DOG1 in GISTs was demonstrated both by immunodetection of the protein and by in situ hybridization. DOG1 immunoreactivity was assessed on two soft tissue tumor microarrays representing 587 soft tissue tumors, including 149 GISTs. 98.7% of scorable GISTs demonstrated immunoreactivity with DOG1 antisera. Only four KIT-negative, non-GIST soft tissue tumors were DOG1 immunoreactive. Several GISTs with mutations in the PDGRFA gene were found to react only by in situ hybridization for DOG1 and to be negative for DOG1 by immunohistochemistry. Future studies are necessary to determine whether monoclonal antibodies against purified DOG1 might yield tools with sensitivity similar to

that seen with in situ hybridization probes. We also confirm PDGFRA expression in a subset of GISTs using in situ hybridization. PDGRFA expression and KIT expression are not mutually exclusive. A subset of KIT-mutated GISTs expresses PDGRFA in addition to KIT while a subset of PDGRFA-mutated tumors also expresses KIT. These data were seen with both immunohistochemical and in situ hybridization techniques.

In addition to the marked similarity in reactivity for DOG1 protein on non-GIST sarcomas, DOG1 protein can also be seen in a subset of melanomas and germ cell tumors as has been described for KIT (West et al., in preparation). Furthermore just as seen with the KIT molecule, a variety of carcinomas also express DOG1. These tumors mostly overlap with the KIT positive tumors. While within the field of soft tissue tumors DOG1 expression appears quite specific for GIST, in a differential diagnostic setting DOG1 reactivity does not exclude carcinomas. Therefore additional markers such as keratin stains should be performed when the differential diagnosis includes carcinoma.

We also demonstrated the feasibility of assessing GIST markers by in situ hybridization on paraffin embedded tissue. Correlation between immunohistochemistry and ISH for DOG1 on GISTs was excellent. In the case of KIT, the correlation was not as strong due to relatively weak or absent ISH signals in some CD117-positive GISTs. It is likely that this reflects lower sensitivity of the *KIT* ISH assay, although cross-reactivity of the CD117 antibody to another epitope on GISTs has not been excluded. In situ hybridization for *PDGFRA* proved to be valuable in identifying KIT-negative GISTs, although DOG1 immunohistochemistry was equally sensitive for these cases. Overall, we have found that ISH techniques are complementary to IHC tests in the evaluation of GISTs.

DOG1 has been recently identified as a gene in the *CCND1-EMS1* locus on human chromosome 11q13, which is amplified in esophageal cancer, bladder tumors, and breast cancer [26]. Human DOG1 protein showed 89.8% total-amino-acid identity with mouse DOG1 protein, and also 58.4%, 38.3%, and 38.6% identity with human C12orf3, C11orf25, and FLJ34272/BAC03704 proteins, respectively. Sequence analysis predicts the presence of eight transmembrane spanning segments. This correlates with our observations of the immunohistochemical localization to the cell membrane. *DOG1* may be part of an as yet unclassified ion transporter family.

Since the biologic function is unknown, it is unclear why DOG1 is so widely expressed in GISTs. Two broad possibilities exist. It may be that the protein has a role in receptor kinase type III signal transduction pathways. On the other hand, DOG1 may be a fortuitous marker of the GIST phenotype, with no direct connection to the KIT and PDGFRA signaling pathways. The finding that mast cells are also immunoreactive for DOG1 tends to favor the former possibility.

In summary, we demonstrate that detection of a novel gene, *DOG1*, identifies the vast majority of both *KIT*- and *PDGFRA*- mutated GISTs. This may be of clinical value in identifying candidates for Gleevec therapy. As a cell membrane associated protein, with markedly elevated expression in GISTs, DOG1 may also be a potential therapeutic target.

ACKNOWLEDGEMENTS:

This work was supported by NIH grants CA85129 and CA84967 and the Howard Hughes Medical Institute. P.O.B. is an Associate Investigator of the Howard Hughes Medical

Institute.

Figure Legends.

Figure 1: Gene array measurement of *KIT* and *DOG1* mRNA expression in 30 soft tissue tumors. Red indicates a relatively high level of expression while green denotes a low level of expression. Gene array data for STTs 524, 629, 417, 418, 219, 111, 656, 94, 335, 794, 1148, 850, 616, 710, 523, 526, 740, 607, and 1220 have been previously reported[18].

Figure 2: Hierarchical clustering of CD117 IHC, *CD117* ISH, *PDGFRA* ISH, DOG1 IHC, and *DOG1* ISH. The results for GISTs on the 2 TMAs have been combined. Antisera or hybridization probes are in columns, tumors in rows. Bright red denotes strong reactivity, while dark red and green indicate low and absent reactivity, respectively. White means missing data.

Figure 3: Staining results on GISTs for CD117 IHC, *CD117* ISH, *PDGFRA* ISH, DOG1 IHC, and *DOG1* ISH in graphic form (see also Table 1).

Figure 4: Immunohistochemical staining with anti-DOG1 serum (S284) and KIT on 2 GISTs (TMA 822 (A) and 3688 (B)) and a synovial sarcoma (TMA 856 (C)).

Figure 5: In situ hybridization of a GIST and leiomyosarcoma with antisense probes to *DOG1* and *KIT* on a GIST and a leiomyosarcoma (LMS). The corresponding negative control sense probes are included in the inset in the upper right hand corner of the GIST sample.

Figure 6: In situ hybridization of *KIT, DOG1*, and *PDGFRA* with GISTs.

A) GIST with mutation in *KIT* shows positive ISH for *KIT, DOG1* but not *PDGFRA*.

B) GIST with mutation in *PDGFRA* shows positive ISH for *DOG1* and *PDGFRA* but not for *KIT*.

C) Negative control leiomyosarcoma.

Tables

Table 1: Staining results for CD117 IHC, *CD117* ISH, *PDGFRA* ISH, DOG1 IHC, and *DOG1* ISH in tabular form (see also Figure 3).

Table 1

	CD117	CD117 ISH	PDGFRA ISH	DOG1	DOG1 ISH	
wt	14	10	9	14	3	total scorables
	14	9	1	14	3	total positive
	100	90	11	100	100	% positive
KIT ex 9	9	7	7	9	6	total scorables
	9	6	2	8	5	total positive
	100	86	29	89	83	% positive
KIT ex 11	86	57	51	81	39	total scorables
	82	47	6	81	38	total positive
	95	82	12	100	97	% positive
KIT ex 13	3	3	2	3	2	total scorables
	3	2	1	3	2	total positive
	100	67	50	100	100	% positive
KIT ex 17	1	1	1	1	0	total scorables
	1	1	0	1	0	total positive
	100	100	0	100	NA	% positive
PDGFRA	8	7	6	8	7	total scorables
	3	1	5	8	5	total positive
	37.5	14	83	100	71	% positive
unknown	23	23	21	23	23	total scorables
	22	21	8	21	22	total positive
	96	91	38	91	96	% positive

References:

1. Weiss SW, Goldblum JR: Soft tissue tumors. St Louis, Mosby, 2001
2. Heinrich MC, Corless C, Demetri GD, Blanke C, von Mehren M, Joensuu H, McGreevey L, Chen CJ, Van den Abbeele A, Druker B, Kiese B, Eisenberg B, Roberts P, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA: Kinase Mutations and Imatinib Response in Patients with Metastatic Gastrointestinal Stronal Tumor. J Clin Oncol 2003, 21:4342-4349
3. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M, Silberman S, Nielsen OS, Group EOfRaToCSTaBS: Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 2001, 358:1421-1423
4. Demetri G, von Mehren M, Blanke C, Van den Abbeele A, Eisenberg B, Roberts P, Heinrich M, Tuveson D, Singer S, Janicek M, Fletcher J, Silverman S, Silberman S, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker B, Corless C, Fletcher C, Joensuu H: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002, 347:472-480
5. Joensuu H, Roberts P, Sarlomo-Rikala M, Andersson L, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri G: Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 2001, 344:1052-1056
6. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW: Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol 2002, 33:459-465
7. van de Rijn M, Rouse RV: CD34: a review. Applied Immunohistochemistry 1994, 2:71-80
8. van de Rijn M, Hendrickson MR, Rouse RV: CD34 expression by gastrointestinal tract stromal tumors. Hum Pathol 1994, 25:766-771
9. Yantiss RK, Spiro IJ, Compton CC, Rosenberg AE: Gastrointestinal stromal tumor versus intra-abdominal fibromatosis of the bowel wall: a clinically important differential diagnosis. Am J Surg Pathol 2000, 24:947-957
10. Smithey BE, Pappo AS, Hill DA: C-kit expression in pediatric solid tumors: a comparative immunohistochemical study. Am J Surg Pathol 2002, 26:486-492
11. Hirota S, Nishida T, Isozaki K, Taniguchi M, Nakamura J, Okazaki T, Kitamura Y: Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. J Pathol 2001, 193:505-510
12. Rubin B, Singer S, Tsao C, Duensing A, Lux M, Ruiz R, Hibbard M, Chen C, Xiao S, Tuveson D, Demetri G, Fletcher C, Fletcher J: KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 2001, 61:8118-8121

13. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA: PDGFRA activating mutations in gastrointestinal stromal tumors. Science 2003, 299:708-710
14. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y: Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology 2003, 125:660-667
15. Bauer S, Corless C, Heinrich M, Dirsch O, Antoch G, Kanja J, Seeber S, Schutte J: Response to imatinib mesylate of a gastrointestinal stromal tumor with very low expression of KIT. Cancer Chemother Pharmacol 2003, 51:261-265
16. Allander SV, Nupponen NN, Ringner M, Hostetter G, Maher GW, Goldberger N, Chen Y, Carpten J, Elkahloun AG, Meltzer PS: Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. Cancer Res 2001, 61:8624-8628
17. Khan J, Wei JS, Ringner M, Saal LH, Ladanyi M, Westermann F, Berthold F, Schwab M, Antonescu CR, Peterson C, Meltzer PS: Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nat Med 2001, 7:673-679
18. Nielsen TO, West RB, Linn SC, Alter O, Knowling MA, O'Connell JX, Zhu S, Fero M, Sherlock G, Pollack JR, Brown PO, Botstein D, van de Rijn M: Molecular characterisation of soft tissue tumours: a gene expression study. Lancet 2002, 359:1301-1307
19. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. 1998 1998, 4:844-847
20. West RB, Harvell J, Linn S, Lui C, Prapong W, Hernandez-Boussard T, Montgomery K, Nielsen TO, Rubin BP, Patel R, Goldblum JR, Brown P, van de Rijn M: Apo D in Soft Tissue Tumors: a Novel Marker for Dermatofibrosarcoma Protuberans. Am J Surg Pathol In press
21. Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Kochli O, Mross F, Dieterich H, Moch H, Mihatsch M, Kallioniemi O, Sauter G: Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001, 159:2249-2256
22. Corless C, McGreevey L, Haley A, Town A, Heinrich M: KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am J Pathol 2002, 160:1567-1572
23. St Croix B, Rago C, Velculescu V, Traverso G, Romans K, Montgomery E, Lal A, Riggins G, Lengauer C, Vogelstein B, Kinzler K: Genes expressed in human tumor endothelium. Science 2000, 289:1197-1202
24. Iacobuzio-Donahue CA, Ryu B, Hruban RH, Kern SE: Exploring the host desmoplastic response to pancreatic carcinoma: gene expression of stromal and neoplastic cells at the site of primary invasion. Am J Pathol 2002, 160:91-99
25. Liu CL, Prapong W, Natkunam Y, Alizadeh A, Montgomery K, Gilks CB, van de Rijn M: Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. Am J Pathol 2002, 161:1557-1565

26. Katoh M, Katoh M: FLJ10261 gene, located within the CCND1-EMS1 locus on human chromosome 11q13, encodes the eight-transmembrane protein homologous to C12orf3, C11orf25 and FLJ34272 gene products. Int J Oncol 2003, 22:1375-1381

Figure 1

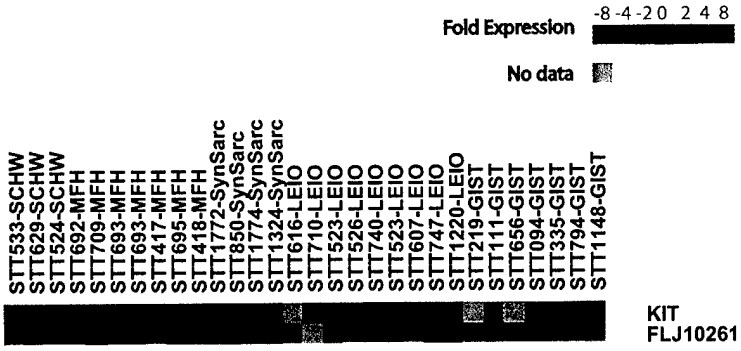

Fold Expression
-8 -4 -2 0 2 4 8
No data
STT533-SCHW
STT629-SCHW
STT524-SCHW
STT692-MFH
STT709-MFH
STT693-MFH
STT693-MFH
STT417-MFH
STT695-MFH
STT418-MFH
STT1772-SynSarc
STT850-SynSarc
STT1774-SynSarc
STT1324-SynSarc
STT616-LEIO
STT710-LEIO
STT523-LEIO
STT526-LEIO
STT740-LEIO
STT523-LEIO
STT607-LEIO
STT747-LEIO
STT1220-LEIO
STT219-GIST
STT111-GIST
STT656-GIST
STT094-GIST
STT335-GIST
STT794-GIST
STT1148-GIST
KIT
FLJ10261

Figure 2

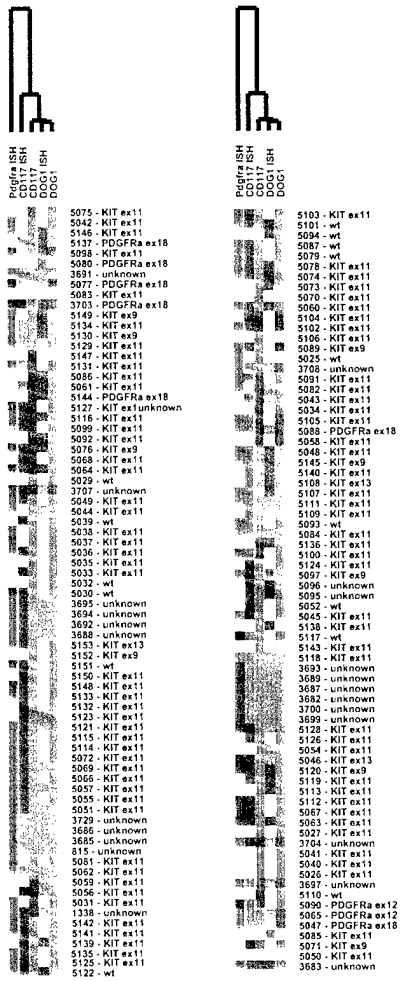

Pdgfra ISH
CD117 ISH
CD117
DOG1
DOG1 ISH
5075 - KIT ex11
5042 - KIT ex11
5146 - KIT ex11
5137 - PDGFRa ex18
5098 - KIT ex11
5080 - PDGFRa ex18
3691 - unknown
5077 - PDGFRa ex18
5083 - KIT ex11
3703 - PDGFRa ex18
5149 - KIT ex9
5134 - KIT ex11
5130 - KIT ex9
5129 - KIT ex11
5147 - KIT ex11
5131 - KIT ex11
5086 - KIT ex11
5061 - KIT ex11
5144 - PDGFRa ex18
5127 - KIT ex1unknown
5116 - KIT ex11
5099 - KIT ex11
5092 - KIT ex11
5076 - KIT ex9
5068 - KIT ex11
5064 - KIT ex11
5029 - wt
3707 - unknown
5049 - KIT ex11
5044 - KIT ex11
5039 - wt
5038 - KIT ex11
5037 - KIT ex11
5036 - KIT ex11
5035 - KIT ex11
5033 - KIT ex11
5032 - wt
5030 - wt
3695 - unknown
3694 - unknown
3692 - unknown
3688 - unknown
5153 - KIT ex13
5152 - KIT ex9
5151 - wt
5150 - KIT ex11
5148 - KIT ex11
5133 - KIT ex11
5132 - KIT ex11
5123 - KIT ex11
5121 - KIT ex11
5115 - KIT ex11
5114 - KIT ex11
5072 - KIT ex11
5069 - KIT ex11
5066 - KIT ex11
5057 - KIT ex11
5055 - KIT ex11
5051 - KIT ex11
3729 - unknown
3686 - unknown
3685 - unknown
815 - unknown
5081 - KIT ex11
5062 - KIT ex11
5059 - KIT ex11
5056 - KIT ex11
5031 - KIT ex11
1338 - unknown
5142 - KIT ex11
5141 - KIT ex11
5139 - KIT ex11
5135 - KIT ex11
5125 - KIT ex11
5122 - wt
Pdgfra ISH
CD117 ISH
CD117
DOG1
DOG1 ISH
5103 - KIT ex11
5101 - wt
5094 - wt
5087 - wt
5079 - wt
5078 - KIT ex11
5074 - KIT ex11
5073 - KIT ex11
5070 - KIT ex11
5060 - KIT ex11
5104 - KIT ex11
5102 - KIT ex11
5106 - KIT ex11
5089 - KIT ex9
5025 - wt
3708 - unknown
5091 - KIT ex11
5082 - KIT ex11
5043 - KIT ex11
5034 - KIT ex11
5105 - KIT ex11
5088 - PDGFRa ex18
5058 - KIT ex11
5048 - KIT ex11
5145 - KIT ex9
5140 - KIT ex11
5108 - KIT ex13
5107 - KIT ex11
5111 - KIT ex11
5109 - KIT ex11
5093 - wt
5084 - KIT ex11
5136 - KIT ex11
5100 - KIT ex11
5124 - KIT ex11
5097 - KIT ex9
5096 - unknown
5095 - unknown
5052 - wt
5045 - KIT ex11
5138 - KIT ex11
5117 - wt
5143 - KIT ex11
5118 - KIT ex11
3693 - unknown
3689 - unknown
3687 - unknown
3682 - unknown
3700 - unknown
3699 - unknown
5128 - KIT ex11
5126 - KIT ex11
5054 - KIT ex11
5046 - KIT ex13
5120 - KIT ex9
5119 - KIT ex11
5113 - KIT ex11
5112 - KIT ex11
5067 - KIT ex11
5063 - KIT ex11
5027 - KIT ex11
3704 - unknown
5041 - KIT ex11
5040 - KIT ex11
5026 - KIT ex11
3697 - unknown
5110 - wt
5090 - PDGFRa ex12
5065 - PDGFRa ex12
5047 - PDGFRa ex18
5085 - KIT ex11
5071 - KIT ex9
5050 - KIT ex11
3683 - unknown

Figure 3

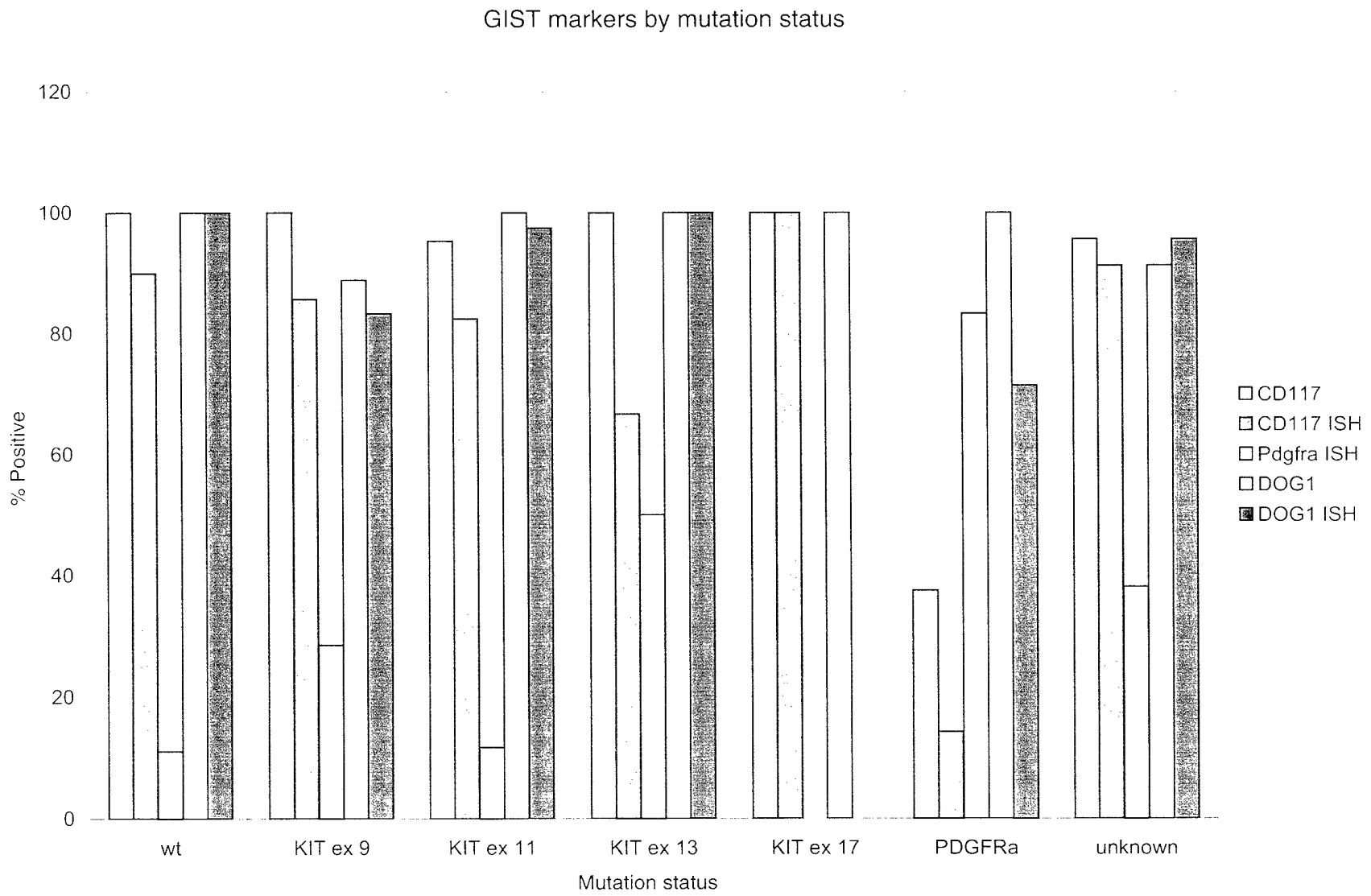

GIST markers by mutation status
120
100
80
60
40
20
0
% Positive
wt
KIT ex 9
KIT ex 11
KIT ex 13
KIT ex 17
PDGFRa
unknown
Mutation status
CD117
CD117 ISH
Pdgfra ISH
DOG1
DOG1 ISH

Figure 4

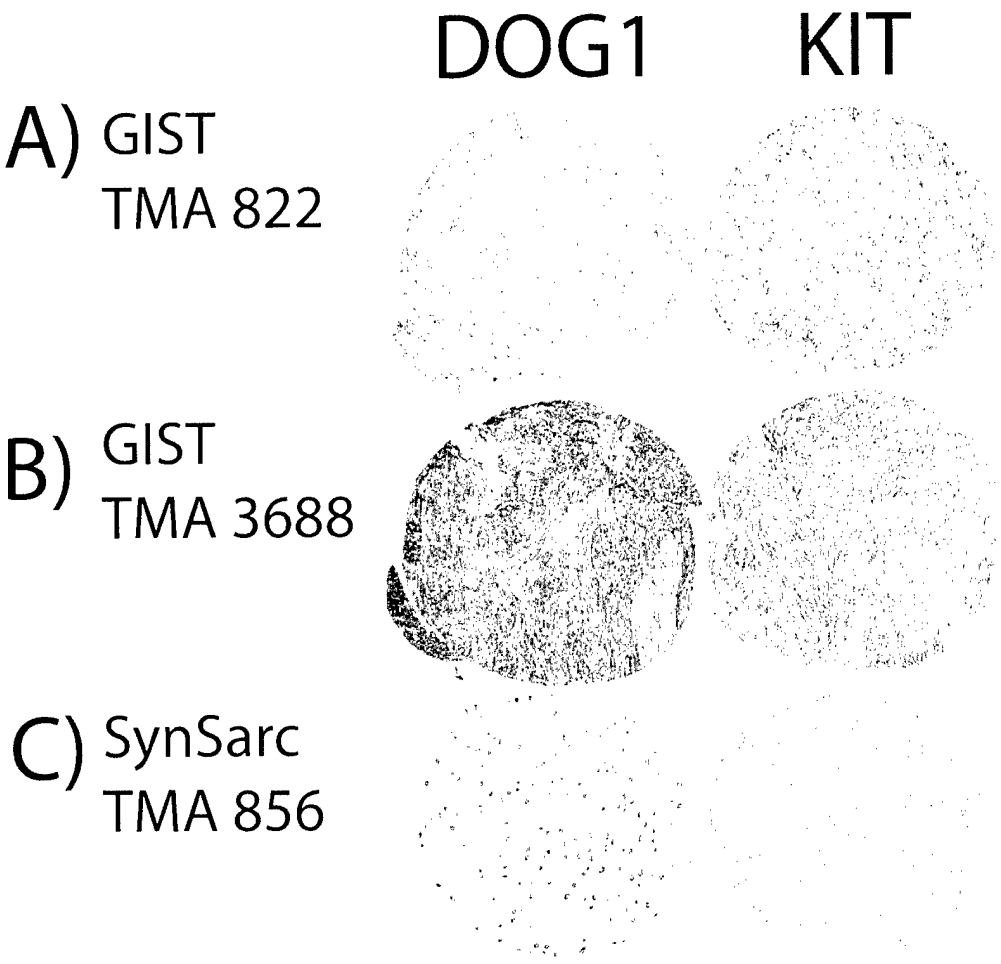


Figure 5

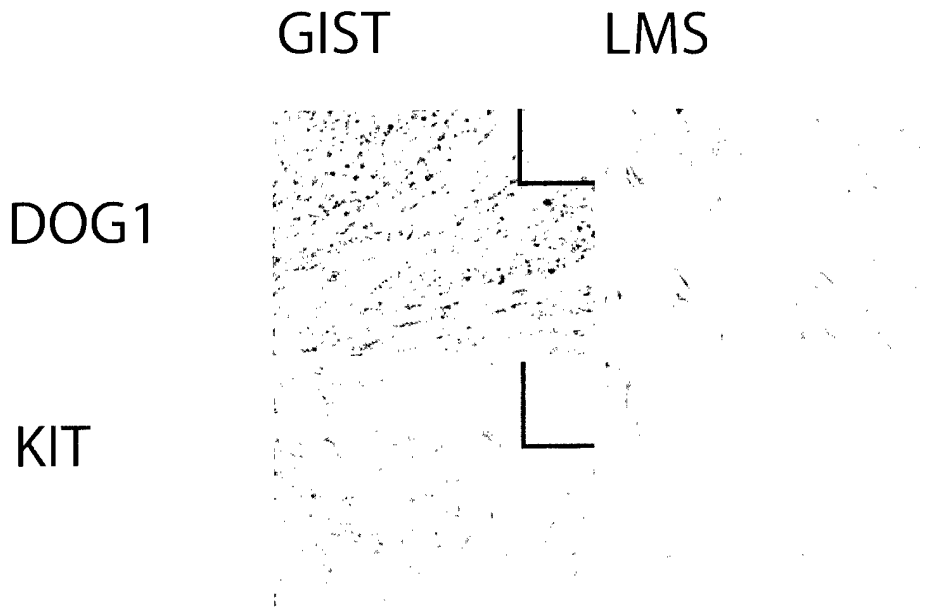
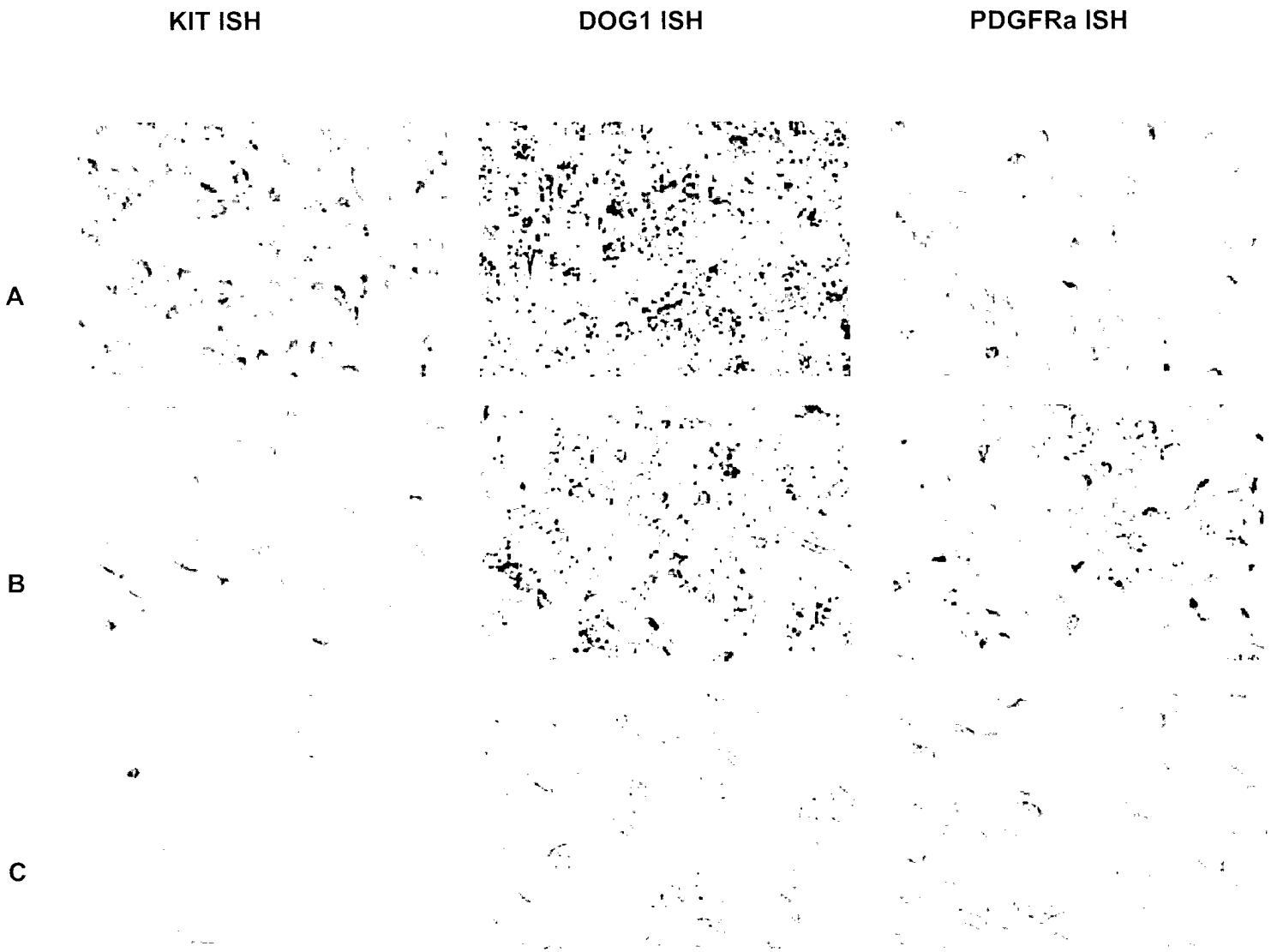


Figure 6



CURRICULUM VITAE

Matt van de Rijn, M.D., Ph.D.

PERSONAL DATA

Name:	Jan Matthijs van de Rijn
Present address:	141 Rocky Creek Road, La Honda, CA 94020
Citizenship:	The Netherlands
Visa status:	Resident Alien
Medical licensure:	State of California: A48323, 6/90
	State of Pennsylvania: MD 055043-L, 8/95
Board certification:	Anatomic Pathology, 11/93

EDUCATION AND APPOINTMENTS

1974-1979:	Medical student at the University of Amsterdam Received Master's degree in Medicine
1/80-1/82:	Graduate student at The Netherlands Cancer Institute Amsterdam. Dr. Jo Hilgers, adviser
1/82-6/84:	Visiting graduate student at the Dana-Farber Cancer Institute Harvard Medical School, Boston. Dr. Cox Terhorst, adviser
12/84:	Received Ph.D., University of Amsterdam Dr. Piet Borst, thesis supervisor Field of study: Protein chemistry of T lymphocyte cell surface antigens
1/85-10/86:	Clinical rotations at the Academic Medical Hospital University of Amsterdam
7/86:	Passed ECFMG (Educational Commission for Foreign Medical Graduates) exam
10/86:	Received M.D., University of Amsterdam
11/86-2/89:	Postdoctoral fellowship in the laboratory of Dr. I. L. Weissman Department of Pathology, Stanford University First year funded by a grant from The Netherlands Organization for the Advancement of Pure Research

From November 87 funded by a postdoctoral grant from the

Multiple Sclerosis Society
Field of study: Protein chemistry and genetic analysis of lymphocyte homing receptors

2/89-6/91:	Residency in pathology at Stanford University Medical Center
7/91-6/92:	Fellowship in surgical pathology, Department of Pathology Stanford University Medical Center
	Co-chief resident in surgical pathology, Department of Pathology, Stanford University Medical Center
7/92-6/93:	Fellowship in immunopathology, Department of Pathology Stanford University Medical Center
7/93-6/94:	Clinical Fellow, Department of Pathology Stanford University Medical Center
7/94-6/95:	Clinical Assistant Professor, Department of Pathology Stanford University Medical Center
7/95-6/98:	Assistant Professor, Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center
7/98-6/2001:	Assistant Professor, Department of Pathology Stanford University Medical Center.
7/2001-present	Associate Professor, Department of Pathology Stanford University Medical Center.

MEMBERSHIPS

United States and Canadian Academy of Pathology
South Bay Pathology Society
American Society for Investigative Pathology
American Association for Cancer Research

Ad hoc referee for:

American Journal of Clinical Pathology
American Journal of Pathology
Blood
Medical and Pediatric Oncology
Southern Medical Journal
The Journal of Histochemistry and Cytochemistry
Cancer

Modern Pathology
British Journal of Cancer
Cell Biochemistry and Biophysics
The Lancet
New England Journal of Medicine
Clinical Cancer Research

NIH/NCI GRANT REVIEWS, WORKING GROUPS, NATIONAL PANELS

Reviewer for RFA: "Technologies for gene expression in the nervous system" July 29-30, 1999, Washington, D.C.

Participant and presenter at NCI "Working Group Meeting on Tissue Arrays." December 16-17, 1999, Rockville, MD.

Participant NCI "State of the Science Sarcoma" meeting June 17-18, 2002, Bethesda, MA

Participant and co-chair of breakout session at NCI "Sarcoma Progress Review Group Roundtable Meeting". October 8-10, 2003, Philadelphia, PA

Member site-visit committee at Erasmus Postgraduate School of Molecular Medicine, November 12-14, 2003, Rotterdam, The Netherlands.

EDITORIAL BOARDS

2003	PLOS: Public Library of Science
2004	The American Journal of Pathology

COURSES/MEETINGS

1. The use of molecular biology and immunohistochemistry in the differential diagnosis of soft tissue tumors (with Fred G. Barr, M.D., Ph.D.), April 1998, American Society of Clinical Pathologists, Los Angeles, CA.

2. The use of molecular biology and immunohistochemistry in the differential diagnosis of soft tissue tumors (with Fred G. Barr, M.D., Ph.D.), April 2000, American Society of Clinical Pathologists, Boston, MA.

3. Co-director and speaker at the 2002 Special Course for Advanced Molecular Pathology at the 91st annual meeting of the United States and Canadian Academy of Pathology, Chicago, IL.

4. Co-chair Proferred Papers section: Bone and Soft Tissue Tumors at the 93rd annual meeting of the United States and Canadian Academy of Pathology, Vancouver, Canada.

5. Co-director of the 2004 Special Course for Advanced Molecular Pathology at the 93rd annual meeting of he United States and Canadian Academy of Pathology, Vancouver, Canada.

INVITED LECTURES

1. EBV positive lymphomas. The Netherlands Cancer Institute, October 1994, Amsterdam, The Netherlands.

2. Use of anti-CD34 antibodies in surgical pathology. January 1995, Systemics Inc., Palo Alto, CA.

3. Use of CD34 in immunohistochemistry. Current Concepts in Surgical Pathology, September 1995, Stanford University, Stanford, CA.

4. Mechanisms of molecular techniques in diagnostic surgical pathology. Current concepts in surgical pathology, September 1995, Stanford University, Stanford, CA.

5. The diagnosis and molecular analysis of soft tissue tumors. (with Fred Barr, M.D., Ph.D.) Pathology Society of Philadelphia, October 1996, Philadelphia, PA.

6. Grand Rounds. Chromosomal translocations in soft tissue tumors. Department of Pathology, Cornell University Medical School, March 1998, New York, NY.

7. Chromosomal translocations in small round cell tumors. Department of Pathology, University of Leiden Medical School, April 1998, The Netherlands.

8. Gene microarray analysis on human breast carcinoma. The Netherlands Cancer Institute, May 1999, Amsterdam, The Netherlands.

9. Poorly differentiated synovial sarcoma. Current Issues in Anatomic Pathology. UCSF-Stanford University, May 1999, San Francisco, CA.

10. Chairman of session "Functional Genomics" and talk "Western blot analysis and RNA expression" at Advances in Laser Capture Microdissection, June 1999, NIH, Bethesda, MD.

11. Keynote address. Towards genomic scale analysis of gene expression in human cancer. European Meeting on Molecular Diagnostics, October 15, 1999, Scheveningen, The Netherlands.

12. Towards genomic scale analysis of gene expression in human cancer. Department of Pathology, University of Pennsylvania, December 14, 1999, Philadelphia, PA.

13. Combining laser capture microdissection and tissue arrays with gene microarray studies. Eos Biotechnology, February 24, 2000, San Francisco, CA.

14. Combining laser capture microdissection and tissue arrays with gene microarray studies. Research Genetics, May 22, 2000, Huntsville, AL.

15. Combining laser capture microdissection and tissue arrays with gene microarray studies. Smith-Kline Beecham Laboratories, May 23, 2000, King of Prussia, PA.

16. Study of human tumors using cDNA and tissue microarrays. Vanderbilt University, November 2000, Nashville, TN.

17. Study of human tumors using cDNA and tissue microarrays. University of British Columbia, December 2000, Vancouver, BC.

18. Study of human tumors using cDNA and tissue microarrays. DNAX, Research Institute, January 2001, Palo Alto, CA.

19. Study of soft tissue tumors and lymphomas using cDNA and tissue microarrays. Colorado Society of Pathology, March 22, 2001, Denver, CO.

20. Studying breast carcinoma using cDNA arrays and tissue arrays. Current Issues in Anatomic Pathology. UCSF-Stanford University, May 24, 2001, San Francisco, CA.

21. Cluster analysis of tissue microarray data. Workshop Tissue Microarray Infostructure. Automated Information Management in the Clinical Laboratory Symposium. Ann Arbor, MI, May 30, 2001.

22. Study of human tumors using cDNA and tissue microarrays. Genentech, San Francisco, CA, June 18, 2001.

23. Study of breast carcinoma and sarcomas using cDNA and tissue microarrays. Cottage Hospital, Santa Barbara, CA, September 20, 2001.

24. Studying human sarcomas using gene and tissue microarrays. University of Washington, Seattle, WA, October 5, 2001.

25. Keynote Address. Studying human neoplasms using cDNA and tissue microarrays. 6th Annual Molecular Medicine Day. Rotterdam, The Netherlands, December 14, 2001.

26. Plenary Lecture. Combining gene array and tissue microarray analysis on human tumors. Symposium "Tumor Profiling", American Association of Cancer Research. San Francisco, May 9, 2002.

27. Plenary Lecture: Expression profiling of tumors. 63rd Annual meeting, Society of Investigative Dermatology. Los Angeles, May 16, 2002.

28. Studying soft tissue tumors with gene and tissue microarrays. Current Issues in Anatomic Pathology. UCSF-Stanford University, May 24, 2002, San Francisco, CA.

29. Understanding breast carcinoma using the tools of molecular biology. Breast Pathology: Current concepts and controversies. June 4, 2002, Harvard Medical School, Boston, MA.

30. Data management for tissue microarrays. October 4, 2002, John Radcliffe Hospital, Oxford University, Great Britain.

31. Expression profiling of sarcomas. October 9, 2002, 24th International Congress of the International Academy of Pathology, Amsterdam, The Netherlands.

32. Keynote Address: The use of gene arrays and tissue microarrays for the study of human tumours. October 9, 2002, 24th International Congress of the International Academy of Pathology, Amsterdam, The Netherlands.

33. The use of gene expression arrays and high density tissue arrays in the study of lymphoid malignancies. October 10, 2002, 24th International Congress of the International Academy of Pathology, Amsterdam, The Netherlands.

34. Studying sarcomas with gene expression profiling and tissue microarrays. October 11, 2002, Leiden University Medical School, Leiden, The Netherlands.

35. Studying sarcomas with high throughput techniques. South-Western Oncology Group (SWOG) Meeting. October 28, 2002, San Antonio, TX.

36. Gene Expression Profiling and Comparative Genomic Hybridization Studies on Sarcomas Using Microarrays. October 31, 2002, Queens University, Kingston, Ontario, Canada.

37. Using tissue microarrays in high throughput studies. Director's Challenge meeting, NCI. November 7, 2002, Bethesda, MA.

38. Hardware and software used in gene microarray analysis. 7th ADNAT meeting, CCMB Research Center, March 1, 2003, Hyderabad, India.

39. Tissue microarrays. 7th ADNAT meeting, CCMB Research Center, March 3, 2003, Hyderabad, India.

40. A demonstration of the Stanford Microarray Database. 7th ADNAT meeting, CCMB Research Center, March 6, 2003, Hyderabad, India.

41. High throughput studies on sarcomas. 7th ADNAT meeting, CCMB Research Center, March 7, 2003, Hyderabad, India.

42. Array based comparative genomic hybridization and laser capture microdissection. 7th ADNAT meeting, CCMB Research Center, March 8, 2003, Hyderabad, India.

43. Expression profiling, comparative genomic hybridization and tissue microarray studies on sarcomas. Dept of Pathology, Virginia Commonwealth University, March 21, 2003, Richmond, VA.

44. Study of human soft tissue tumors using cDNA and tissue microarrays. FASEB, American Society for Investigative Pathology. April 12, 2003, San Diego, CA.

45. Studying breast carcinoma using the tools of molecular biology. Breast Pathology: Current concepts and controversies. June 2, 2003, Harvard Medical School, Boston, MA.

46. Application of microarray technology in diagnostic pathology (with Blake Gilks, MD). 54th Annual Meeting of the Canadian Association of Pathologists. July 8, 2003, Charlottetown, Prince Edward Island, Canada.

47. Gene expression arrays in GIST. 3rd international symposium on GIST. September 19, 2003, Helsinki, Finland.

48. Gene and tissue array studies on human tumors. 2nd Ankara Biotechnology Days. September 22, 2003, Ankara, Turkey.

49. Gene microarrays to profile human tumors. BayChem 2003, Am. Ass. for Clin. Chem. October 3, 2003, Redwood City, CA.

50. Molecular profiles of sarcomas. Soutwest Oncology Group. October 4, 2003, Seattle, WA.

51. Data analysis for tissue microarrays. October 20, 2003, Centro National de Investigaciones Oncologicas (CNIO), Madrid, Spain.

52. Gene and tissue microarray studies on sarcomas. October 22, 2003, Hospital Sant Pau, Barcelona, Spain.

53. Grand Rounds. Gene and tissue microarray studies on sarcomas. November 3, 2003, Department of Pathology, Brigham and Women's Hospital, Boston, MA

54. EGFR expression measured by gene arrays and tissue microarrays in synovial sarcoma. November 10, 2003, University Hospital Leuven, Belgium.

55. Some examples of extension and validation of gene array data by tissue microarray analysis. November 22, 2003, NCI Director's Challenge Meeting, Bethesda, MA.

56. Grand Rounds. Gene and tissue microarray studies on sarcomas. January 28, 2004. Department of Pathology, UCLA Medical Center, Los Angeles, CA.

57. Gene expression profiling of soft tissue tumors. March 7 2004. International Society of Bone and Soft Tissue Pathology at the 93rd Annual meeting United States and Canadian Academy of Pathology, Vancouver, Canada.

58. Grand Rounds. Gene and tissue microarray studies on sarcomas. March 25, 2004. Department of Pathology, University of North Carolina, Chapel Hill, NC.

59. Applications of gene and tissue microarrays to the diagnosis and classification of sarcomas. March 31, 2004. Sarcoma Diagnosis and Treatment Session at the 95th annual meeting of the American Association of Cancer Research. Orlando, FL.

60. Applications of gene and tissue microarrays to pathology. May 6, 2004. Leopoldina Meeting: Pathologie im Wandel. Rotach-Egern, Germany.

LECTURES at STANFORD UNIVERSITY MEDICAL CENTER

1. Laser capture microdissection and gene microarray analysis. Department of Medicine, February 23, 1999.

2. Gene microarray analysis of human breast carcinomas and laser capture microdissection. Surgery grand rounds, with S. Jeffrey and C. Barry, March 24, 1999.

3. Cancer profiling. Lecture in Biomedical Genomics course, org. Bob Shafer, March 6, 2002.

4. Gene arrays. Medical student talk, Pathology 230 course. April 16, 2003

5. Use of cDNA and tissue microarrays for studies on human tumors. Lecture in Biomedical Genomics course, org. Bob Shafer, February 4, 2004.

PUBLICATIONS

PEER REVIEWED PUBLICATIONS

1. M. van de Rijn, A.H.M. Geurts van Kessel, V. Kroezen, A.J. van Agthoven, K. Versteijnen, C. Terhorst, and J. Hilgers. (1983) Localization of a gene controlling the expression of the human transferrin receptor to the region q12-qter of chromosome 3. *Cytogenetics and Cell Genetics* **36**:525-31.

2. M. van de Rijn, P.G. Lerch, R.W. Knowles and C. Terhorst. (1983) The thymic differentiation antigens T6 and M241 are two unusual MHC Class I antigens. *Journal of Immunology* **131**:851-5.

3. P.G. Lerch, M. van de Rijn, P. Schrier and C. Terhorst. (1983) Biochemical comparison of the T6 antigen and the HLA-A,B antigens. *Human Immunology* **6**:13-30.

4. C. Bernabeu, D. Finlay, M. van de Rijn, R.T. Maziarz, P.A. Biro, H. Spits, J.E. de Vries and C. Terhorst. (1983) Expression of the major histocompatibility antigens HLA-A2 and HLA-B7 by DNA mediated gene transfer. *Journal of Immunology* **131**:2032-7.

5. H.J. van der Reijden, E.R. van Wering, M. van de Rijn, C.J.M. Melief, M.B. van't Veer, H. Behrendt and A.E.G.Kr. von dem Borne. (1983) Immunological typing of acute lymphoblastic leukemia. *Scandinavian Journal of Haematology* **30**:356.

6. J.J.M. van Dongen, H. Hooijkaas, K. Hahlen, K. Benne, W.M. Bitter, A.A. van de Linde-Preesman, I.L.M. Tettero, M. van de Rijn, J. Hilgers, G.E. van Zanen and A. Hagemeijer. (1984) Detection of minimal residual disease in Tdt positive T-cell malignancies by double immunofluorescence staining. In: Minimal Residual Disease in Acute Leukemia. (B. Lowenberg, and A. Hagebeek, eds.) Martinus Nijhoff Publishers b. v., The Hague, The Netherlands.

7. M. van de Rijn, P.G. Lerch, B.R. Bronstein, R.W. Knowles, A.K. Bhan and C. Terhorst. (1984) Human cutaneous dendritic cells express two glycoproteins T6 and M241 which are biochemically identical to those found on cortical thymocytes. *Human Immunology* **9**:201-10.

8. C. Bernabeu, M. van de Rijn, P.G. Lerch and C. Terhorst. (1984) β_2-microglobulin from serum associates with MHC Class I antigens on the surface of cultured cells. *Nature* **308**:642-5.

9. M. van de Rijn, C. Bernabeu, B. Royer-Pokora, J. Leiss, J.G. Seidman, H. Spits, J. E. de Vries and C. Terhorst. (1984) Recognition of HLA-A2 by cytotoxic T-lymphocytes after DNA transfer into human and murine cells. *Science* **226**:1083-5.

10. P. Snow, M. van de Rijn and C. Terhorst. (1985) Association of the T8 T-cell differentation antigen with the Class I MHC thymocyte antigen T6 on the thymocyte cell surface. *European Journal of Immunology* **15**:529-32.

11. P.G. Lerch, M. van de Rijn, J.E. Smart, R.W. Knowles and C. Terhorst. (1985) Isolation and purification of the human thymocyte antigens T6 and M241. *Molecular Immunology* **23**:131-9.

12. H. Spits, W. van Schooten, H. Keizer, G. van Seventer, M. van de Rijn, C. Terhorst and J.E. de Vries. (1986) Evidence that recognition of alloantigen by the T-cell receptor of allospecific cytotoxic T-cell clones (CTL) has to be preceded by antigen nonspecific conjugate formation between the CTL clone and target cells. *Science* **232**:403-5.

13. M. Siegelman, M. van de Rijn, and I.L. Weissman. (1989) Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science* **243**:1165-72.

14. M. van de Rijn, S. Heimfeld, G.J. Spangrude, and I.L. Weissman. (1989) Mouse hematopoietic stem cell antigen Sca-1 is a member of the Ly-6 antigen family. *Proceedings of the National Academy of Sciences* **86**:4634-8.

15. M. van de Rijn, I.L. Weissman, and M. Siegelman. (1990) Biosynthesis pathway of gp90^{MEL-14}, the mouse lymph node-specific homing receptor. *Journal of Immunology* **145**:1477-82.

16. M. van de Rijn, D.P. Regula, Jr., and M. Billingham. (1990) Autopsy findings after coronary rotational atherectomy. *American Journal of Cardiovascular Pathology* **3**:301-4.

17. O. Kamel, M. van de Rijn, L. Weiss, G. Del Zoppo, P. Hench, B. Robbins, P. Montgomery, R. Warnke, R. Dorfman. (1993) Reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. *New England Journal of Medicine* **328**:1317-21.

18. M. van de Rijn, M.R. Hendrickson, R.V. Rouse. (1994) An immunohistochemical study of inflammatory fibroid polyps of the gastrointestinal tract. *Applied Immunohistochemistry* **2**:54-9.

19. B.R. Smoller, M. van de Rijn, D. LeBrun, R.A. Warnke. (1994) *bcl*-2 expression reliably distinguishes trichoepitheliomas from basal cell carcinomas. *British Journal of Dermatology* **131**:28-31.

20. M. van de Rijn, C.M. Lombard, R.V. Rouse. (1994) Expression of CD34 by solitary fibrous tumors of the pleura, mediastinum and lung. *American Journal of Surgical Pathology* **18**:814-20.

21. M.N. Rizeq, M. van de Rijn, M.R. Hendrickson, R.V. Rouse. (1994) A comparative immunohistochemical study of uterine smooth muscle neoplasms with emphasis on the epithelioid variant. *Human Pathology* **25**:671-77.

22. M. van de Rijn, M.R. Hendrickson, R.V. Rouse. (1994) CD34 expression by gastrointestinal tract stromal tumors. *Human Pathology* **25**:766-71.

23. O.W. Kamel, M. van de Rijn, D.P. LeBrun, L.M. Weiss, R.A. Warnke, R.F. Dorfman. (1994) Lymphoid neoplasms in patients with rheumatoid arthritis and dermatomyositis: frequency of Epstein-Barr virus and other features associated with immunosuppression. *Human Pathology* **25**:638-43.

24. V. Bhargava, D. Kell, M. van de Rijn, R.A. Warnke. (1994) *bcl*-2 immunoreactivity in breast carcinoma correlates with hormone receptor positivity. *American Journal of Pathology* **145**:535-40.

25. A.B. Gelb, M. van de Rijn, D.P. Regula, J.P. Cornbleet, O.W. Kamel, D.S. Horoupian, M.L. Cleary, R.A. Warnke. (1994) Epstein-Barr virus associated natural killer-large granular lymphocyte leukemia. *Human Pathology* **25**:953-60.

26. M.V. Dolezal, O.W. Kamel, M. van de Rijn, M.L. Cleary, R.S. Sibley, R.A. Warnke. (1995) Virus-associated hemaphagocytic syndrome characterized by clonal Epstein-Barr virus genome. *American Journal of Clinical Pathology* **103**:189-94.

27. C.R.J. Morales-Ducret, M. van de Rijn, D. LeBrun, B.R. Smoller. (1995) *bcl*-2 in primary malignancies of the skin. *Archives of Dermatology* **131**:909-15.

28. C.R.J. Morales-Ducret, M. van de Rijn, B.R. Smoller. (1995) *bcl*-2 expression in melanocytic nevi: insights into the biology of dermal maturation. *Archives of Dermatology* **131**:915-9.

29. M.M. Hanasono, O.W. Kamel, P.P. Chang, M.N Rizeq , M.E. Billingham, M. van de Rijn. (1995) Detection of Epstein-Barr virus in cardiac biopsies of heart transplant patients with lymphoproliferative disorders. *Transplantation* **60**:471-3.

30. O.W. Kamel, P.P. Chang, F. Hsu, M.V. Dolezal, R.A. Warnke, M. van de Rijn. (1995) Clonal VDJ recombination of IgH gene by PCR in classical Hodgkin's disease. *American Journal of Clinical Pathology* **104**:419-23.

31. D.A. Arber, O.W. Kamel, R.E. Davis, M. van de Rijn, L.M. Weiss. (1995) Frequent presence of the Epstein-Barr virus in inflammatory pseudotumor. *Human Pathology* **26**:1093-8.

32. I. von Luettichau, P.J. Nelson, J.M. Pattison, M. van de Rijn,, P. Hue, C.J. Wiederman, R.A.K. Stahl, R.K. Sibley, A.M. Krensky. (1996) Rantes chemokine expression in diseased and normal human tissues. *Cytokine* **8**:89-98.

33. M. van de Rijn, M.L. Cleary, R.A. Warnke, O.W. Kamel. (1996) Epstein-Barr virus clonality in lymphoma in patients with rheumatoid arthritis. *Arthritis & Rheumatism* **39**:638-42.

34. A.B. Gelb, M. van de Rijn, R.A. Warnke, O.W. Kamel. (1996) Pregnancy associated lymphomas: a clinicopathological study. *Cancer* **78**:304-10.

35. M.E. Rothenberg, R. Ownbey, P. Mehlhop, P.M. Loiselle, M. van de Rijn, J.V. Bonventre, H.C. Oettgen, P. Leder, and A.D. Luster. (1996) Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin-5 in mice. *Molecular Medicine* **2**:334-48.

36. O.W. Kamel, L.M. Weiss, M. van de Rijn, T.V. Colby, D.W. Kingma, E.S. Jaffe. (1996) Hodgkin's disease and Hodgkin's disease-like lymphoproliferative disorders in patients receiving long-term low-dose methotrexate therapy. *American Journal of Surgical Pathology* **20**:1279-87.

37. M. van de Rijn, O.W. Kamel, P.P. Chang, A.L. Lee, R.A. Warnke, and K.E. Salhany. (1997) Primary low grade endometrial B-cell lymphoma. *American Journal of Surgical Pathology* **21**:187-94.

38. P.D. Mehlhop, M. van de Rijn, A.B. Goldberg, V.P. Kurup, T.R. Martin, H.C. Oettgen. (1997) Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur even in the absence of IgE in a mouse model of asthma. *Proceedings of the National Academy of Sciences* **94**:1344-9.

39. M. van de Rijn, V. Bhargava, H. Molina-Kirsch, R. Carlos, R.A. Warnke, and O.W. Kamel. (1997) Epstein-Barr virus (EBV) associated head and neck lymphomas in Guatemala. *Human Pathology* **28**:834-9.

40. M. van de Rijn, F.G. Barr, Q. Xiong, K.E. Salhany, D.L. Fraker, C. Fisher. (1997) Radiation associated synovial sarcoma. *Human Pathology* **28**:1325-28.

41. J.L. Hunt, M. van de Rijn, P.K. Gupta. (1998) Immunohistochemical analysis of gel-transferred cells in cytologic preparations following slide division. *Diagnostic Cytopathology* **18**:377-80.

42. M. van de Rijn, P.D. Mehlhop, A. Judkins, M.E. Rothenberg, A.D. Luster, H.C. Oettgen. (1998) A murine model of allergic rhinitis: studies on the role of IgE in pathogenesis and analysis of the eosinophil influx elicited by allergen and by eotaxin. *Journal of Allergy and Clinical Immunology,* **102**:65-74.

43. A.R. Judkins, K.T. Montone, V.A. Livolsi, M. van de Rijn. (1998) Sensitivity and specificity of antibodies on necrotic tumor tissue. *American Journal of Clinical Pathology,* **110**:641-646.

44. M.P. McLane, A. Haczku, M. van de Rijn, C. Weiss, V. Ferrante, D. McDonald, J. Renauld, N.C. Nicolaides, K.J. Holroyd, R.C. Levitt. (1998) Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway

hyperresponsiveness in transgenic mice. *American Journal of Respiratory Cell and Molecular Biology,* **19**:713-20.

45. M. van de Rijn, F.G. Barr, J. Shipley, Q.B. Xiong, M. Hedges, C. Fisher. (1999) Poorly differentiated synovial sarcoma: an analysis of clinical, pathologic and molecular genetic features. *American Journal of Surgical Pathology,* **23**:106-12.

46. S. Li, J.M. Junkins-Hopkins, D.F. Fraker, M. van de Rijn. (1999) Desmoplastic fibroma (collagenous fibroma): a case report and review of the literature. *International Journal of Surgical Pathology,* 7:39-44.

47. M. van de Rijn, F.G. Barr, Q.B. Xiong, M. Hedges, C. Fisher. (1999) Absence of SYT-SSX fusion products in soft tissue tumors other than synovial sarcoma. *American Journal of Clinical Pathology,* **112**:43-9.

48. A. Krasinkas, M. Pardington, C. Reynolds, M. van de Rijn, K.T. Montone. (1999) Colonic adenocarcinoma presenting as supraclavicular lymphadenopathy in a 26 year old man: a case report. *American Journal of Gastroenterology,* **94**:282-3.

49. C.M. Perou, S.S. Jeffrey, M. van de Rijn, M.B. Eisen, D.T. Ross, A. Pergamenschikov, J.C.F. Lee, D. Lashkari, D. Shalon, P.O. Brown, and D. Botstein. (1999) Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proceedings of the National Academy of Science,* **96**:9212-7.

50. M.E. Sanders, M. van de Rijn, F.G. Barr. (1999) Detection of a variant SYT-SSX1 fusion in a case of predominantly epithelioid synovial sarcoma. *Molecular Diagnosis,* **4**:65-70.

51. O.W. Kamel, E.A. Holly, M. van de Rijn, C. Lele, A. Sah. (1999) A population based, case control study of non-Hodgkin's lymphoma in patients with rheumatoid arthritis. *Journal of Rheumatology,* **26**:1676-80.

52. Y. Natkunam, R.V. Rouse, S. Zhu, C. Fisher, M. van de Rijn. (2000) Immunoblot analysis of CD34 expression in histologically diverse neoplasms. *American Journal of Pathology* **156**:21-7.

53. D.T. Ross, U. Scherf, M.B. Eisen, C.M. Perou, C. Rees, P. Spellman, V. Iyer, S.S. Jeffrey, M. van de Rijn, M. Waltham, A. Pergamenschikov, J.C.F. Lee, D. Lashkari, D. Shalon, T. C. Myers, J. Weinstein, D. Botstein, and P.O. Brown. (2000) Systematic variation in gene expression patterns in human cancer cell lines. *Nature Genetics,* **24**:227-35

54. J. P. Higgins, M. van de Rijn, C. Jones, J. Zehnder, R. A. Warnke. (2000) Peripheral T-cell lymphoma complicated by a proliferation of large B-cells; "large B-cell rich T-cell lymphoma." *American Journal of Clinical Pathology,* **11**:236-47.

55. P.D. Mehlhop, M. van de Rijn, J.P. Brewer, A.B. Kisselgof, R.S. Geha, H.C. Oettgen, T.R. Martin. (2000) CD40L, but not CD40, is required for allergen-induced bronchial hyperresponsiveness in mice. *American Journal of Respiratory Cell and Molecular Biology,* **23**:646-51.

56. C.M. Perou, T. Sorlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J.R. Pollack, D.T. Ross, H. Johnsen, L.A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S. Zhu, P.E. LØnning, A-L. BØrresen-Dale, P.O. Brown, D. Botstein. (2000) Molecular portraits of human breast tumours. *Nature*, **406**:747-752.

57. Y. Natkunam, R.A. Warnke, K. Montgomery, B. Falini, M. van de Rijn. (2001) Analysis of MUM1/IRF4 protein expression utilizing tissue microarrays and immunohistochemistry. *Modern Pathology,* **7**:686-694.

58. J. Goldsmith, M. van de Rijn, N. Syed. (2001) Orbital hemangiopericytoma and solitary fibrous tumor: lesions of similar pathogenesis? *International Journal of Surgical Pathology,* **9**:295.

59. T. Sørlie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorsen, H. Quist, C.A. Rees, P.O. Brown, David Botstein, P.E. Lønning, A. Børresen-Dale (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proceedings of the National Academy of Science*, **98:**10869-74.

60. M.E. Garber, O.G. Troyanskaya, K. Schluens, S. Petersen, Z. Thaesler, M. Pacyna-Gengelbach, M. van de Rijn, M. Rosen, C.M. Perou, G Whyte, R.I. Altman, P.O. Brown, D. Botstein, I. Petersen. (2001) Diversity of gene expression and patient outcome in adenocarcinoma of the lung. *Proceedings of the National Academy of Sciences,* **98**:13784-9.

61. X. Chen, S.T. Cheung, S. So, S.T. Fan, C. Barry, J. Higgins, K. Lai, S. Dudoit, I.O.L. Ng, M. van de Rijn, D. Botstein, P.O. Brown. (2002) Gene expression patterns in human liver cancers. *Molecular Biology of the Cell* **13**:1929-39.

62. T.O. Nielsen, R.B. West, S.C. Linn, O. Alter, M.A. Knowling, J. O'Connell, S. Zhu, M. Fero, G. Sherlock, J.R. Pollack, P.O. Brown, D. Botstein, M. van de Rijn. (2002) Molecular characterization of soft tissue tumors: a gene expression study. *The Lancet,* **359**:1301-7.

63. C.L. Liu, W. Prapong, Y. Natkunam, A.A. Alizadeh, K. Montgomery, B. Gilkes, M. van de Rijn. (2002) Data management for tissue microarrays. *American Journal of Pathology*, **161**:1557-65.

64. A. Alkushi, J. Irving, F. Hsu, B. Dupiuis, C.L. Liu, M. van de Rijn, C. B. Gilks. (2002) Immunoprofile of cervical and endometrial adenocarcinomas using a tissue microarray. *Virchows Archives,* **442**: 271-7.

65. H.Y. Chang, J. Chi, S. Dutoit, C. Bondre, M. van de Rijn, D. Botstein, P.O. Brown. (2002) Topographic differentiation and positional memory in human fibroblasts. *Proceedings of the National Academy of Science,* **99**:12877-82.

66. M. van de Rijn, C. Perou, R. Tibshirani, P. Haas, O. Kallioniemi, J. Kononen, J. Torhorst, G. Sauter, M. Zuber, O. R. Köchli, F. Mross, H. Dieterich, R. Seitz, D. Ross, D. Botstein, P. Brown. (2002) Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. *American Journal of Pathology,* **161**:1991-6.

67. F.D. Hsu, T.O. Nielsen, A. Alkushi, B. Dupuis, D. Huntsman, C.L Liu, M. van de Rijn, C.B. Gilks. (2002) Multiple tumor tissue microarrays are an effective quality assurance control for diagnostic immunohistochemistry. *Modern Pathology,* **15**:1374-80.

68. J.P. Higgins, K. Montgomery, L. Wang, E. Domanay, R. Warnke, J.D. Brooks, M. van de Rijn. (2003) Expression of FKB12 in benign and malignant vascular endothelium: a conventional immunohistochemial and tissue microarray study. *American Journal of Surgical Pathology,* **27**:58-64

69. Z. Fan, M. van de Rijn, K. Montgomery, R.V. Rouse. (2003) Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 613 tumors tested using tissue microarrays and conventional tissue sections. *Modern Pathology,* **16**:137-44.

70. M.N. Storz, M van de Rijn, Y.H. Kim, S. Mraz-Gerhard, S. Kohler. (2003) Application of DNA microarrays in cutaneous B-cell lymphoma. *The Journal of Investigative Dermatology*, **120**: 865-70.

71. J.P. Higgins, R. Shinghal, H. Gill, J. H. Reese, M. Terris, R.J. Cohen, R. Tibshirani, G. Sherlock, M. Fero, J.R. Pollack, P.O. Brown, D. Botstein, M. van de Rijn, J.D. Brooks. (2003) Gene expression patterns in renal cell carcinoma assessed by complementary DNA microarrays. *American Journal of Pathology*, **162:**925-32.

72. I.L. Wapnir, M. van de Rijn, K. Nowels, P.S. Amenta, K. Walton, K. Montgomery, R.S. Greco, O. Dohan, N. Carrasco. (2003) Immunohistochemical profile of the sodium iodide symporter (NIS) in thyroid, breast and other carcinomas using high density tissue microarrays and conventional sections. *Journal of Clinical Endocrinology and Metabolism,* **88:**1880-8.

73. T.O. Nielsen, F.D. Hsu, J.X. O'Connell, C.B. Gilks, R.B. West, C.L. Liu, D. Botstein, P.O. Brown, M. van de Rijn. (2003) Tissue microarray analysis of novel synovial sarcoma immunohistochemical markers. *American Journal of Pathology,* **163:**1449-56.

74. J. Chi, H.Y. Chang, G. Haraldsen, F.L. Jahnsen, O.G. Troyanskaya, D.S. Chang, Z. Wang, S.G. Rockson, M. van de Rijn, D. Botstein, P.O. Brown. (2003) Endothelial cell diversity revealed by global expression profiling. *Proceedings of the National Academy of Science,* **100**:10623-8

77. S.C. Linn, R.B. West, J.R. Pollack, S. Zhu, T. Hernandez-Boussard, T.O. Nielsen, B.P. Rubin, R. Patel, J.R. Goldblum, D. Siegmund, D. Botstein, P.O. Brown, C.B. Gilks, M. van de Rijn. (2003) Gene expression patterns and gene copy number changes in dermatofibrosarcoma protuberans. *American Journal of Pathology,* **163**:2383-95.

76. M.E. Schaner, D.T. Ross, G. Ciaravino, T. Sørlie, O. Troyanskaya, M. Diehn, Y.C. Wang, G.E. Duran, T.L. Sikic, S. Caldeira, H. Skomedal, I. Tu, T. Hernandez-Boussard, S.W. Johnson, P.J. O'Dwyer, M.J. Fero, G.B. Kristensen, A. Børresen-Dale, T. Hastie, R. Tibshirani, M. van de Rijn, N.N. Teng, T.A. Longacre, D. Botstein, P.O. Brown, B.I. Sikic. (2003) Gene expression patterns in ovarian epithelial carcinomas. *Molecular Biology of the Cell,* **14**:4376-86.

77. J.P.T. Higgins, L. Wang, N. Kambham, K. Montgomery, V. Mason, S. Vogelmann, K. Lemley, P.O. Brown, J.D. Brooks, M. van de Rijn. (2004) Gene expression in the normal adult human kidney assessed by complementary DNA microarray. *Molecular Biology of the Cell.* **15**:649-656, *[Epub ahead of print, 2003 Dec 2].*

78. J.D. Harvell, S. Kohler, S. Zhu, T. Hernandez-Boussard, J.R. Pollack, M. van de Rijn. (2004) High resolution array-based comparative genomic hybridization for distinguishing paraffin-embedded Spitz nevi and melanomas. *Diagnostic Molecular Pathology, in press.*

79. H.Y. Chang, J. Sneddon, A. Alizadeh, R. Sood, R. West, M. van de Rijn, D. Botstein, P.O. Brown. (2004) Tumor as wound: prognostic role of fibroblast serum response in human cancer progression. *PLOS Biology, Vol 2, No. 2 E7 DOI:10371.*

80. J. Lapointe, C. Li, M. van de Rijn, J.P. Higgins, E. Bair, K. Montgomery, M. Ferrari, P. Ekman, A.M. DeMarzo, R. Tibshirani, D. Botstein, P.O. Brown, J.D. Brooks, J.R. Pollack. (2004) Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proceedings of the National Academy of Science,* **101**:*811-6.*

81. K. Montgomery, S. Zhao, M. L. Whitfield, M. van de Rijn, Y. Natkunam. A novel method for making tissue microarrays from small numbers of suspension cells. (2004) *Applied Immunohistochemistry and Molecular Morphology, in press.*

82. R.B. West, J. Harvell, S.C. Linn, C.L. Lui, W. Prapong, T. Hernandex-Boussard, K. Montgomery, T.O. Nielsen, B.P. Rubin, R. Patel, J.R. Goldblum, P.O. Brown, M. van de Rijn. (2004) APOD in soft tissue tumors: a novel marker for dermatofibrosarcoma protuberans. *American Journal of Surgical Pathology, in press.*

83. X. Chen, J. Higgins, S. Cheung, R. Ki, V. Mason, K. Montgomery, S. Fan, M. van de Rijn, S. So. (2004) Expression patterns of endothelial cell markers in HCC and non-neoplastic liver. *Modern Pathology, in press.*

84. R.B. West, C.L. Corless, B.P. Rubin, S. Subramanian, K. Montgomery, S. Zhu, C. Ball, T.O. Nielsen, R. Patel, J.R. Goldblum, P.O. Brown, M.C. Heinrich, M. van de Rijn. (2004) The novel marker, DOG1, is expressed ubiquitously in GI stromal tumors irrespective of KIT or PDGFRA mutation status. *American Journal of Pathology, in press.*

85. T.O. Nielsen, F.D. Hsu, K. Jensen, M. Cheang, G. Karaca, T. Hernandez-Boussard, D. Cowan, L. Dressler, C. Livasy, L.A. Akslen, J. Ragaz, A.M. Gown, C.B. Gilks, M. van de Rijn, C.M. Perou. (2004) Immunohistochemical and clinical characterization of a basal cell subtype of invasive breast carcinoma. *Clinical Cancer Research, in press.*

86. N. Makretsov, D.Huntsman, T.O. Nielsen, E. Yorida, M. Peacock, M. Cheang, S. Dunn, M. Hayes, M. van de Rijn, C. Bajdik, C.B. Gilks. (2004) Identification of prognostically significant subgroups in breast carcinoma by clustering of immunostain results. *Clinical Cancer Research, in press.*

REVIEWS

1. C. Terhorst, J. Borst, P. Lerch, M. van de Rijn, P. Snow, H. Spits, J. de Vries. (1983) Structural and functional aspects of the T-cell differentiation antigens T3, T6, and T8. *Hamatologie und Bluttransfus* **28**:440-6.

2. M. van de Rijn, R.V. Rouse. (1994) CD34: a review. *Applied Immunohistochemistry* **2**:71-80.

3. O.W. Kamel, M. van de Rijn, M.M. Hanasono, R.A. Warnke. (1995) Immunosuppression-associated lymphoproliferative disorders in rheumatic patients. *Leukemia and Lymphoma* **16**:363-68.

4. M. van de Rijn, K.E. Salhany. (1997) Lymphoid neoplasms of the thyroid. *Pathology Case Reviews* 218-25.

5. A.A. Alizadeh, D.T. Ross, C.M. Perou, M. van de Rijn. (2001) Towards a novel classification of human malignancies based on gene expression patterns. *The Journal of Pathology*, **195:**41-52.

6. J.R Pollack, M. van de Rijn, D. Botstein. (2002) Challenges in developing a molecular characterization of cancer. *Seminars in Oncology,* **29**:280-5

7. M.van de Rijn, B. Rubin. (2002) Gene expression studies on soft tissue tumors. (Invited Commentary) *American Journal of Pathology,* **161**:1531-4.

8. R.B. West, M. van de Rijn. (2002) Gene expression profiles in sarcoma. *Histopathology*, **41** (Suppl 2):249-55.

9. Y. Natkunam, M. van de Rijn. (2002) The use of gene expression arrays and high density tissue microarrays in the study of hematolyphoid malignancies. *Histopathology*, **41** (Suppl 2): 520-5.

10. S.C. Linn, M. van de Rijn, G. Giaccone. (2003) Improved diagnostics and therapeutics with DNA microarrays. Part I: Technology and data analysis. *Nederlands Tijdschrift voor Geneeskunde,* **147**:795-9.

11. S.C. Linn, M. van de Rijn, G. Giaccone. (2003) Improved diagnostics and therapeutics with DNA microarrays. Part II: Applications. *Nederlands Tijdschrift voor Geneeskunde,* **147**:800-4.

12. M van de Rijn, C.B. Gilks. (2004) Applications of microarrays to histopathology. *Histopathology,* **44**:97-108.

BOOK CHAPTERS

1. S. Linn, R.B. West, M. van de Rijn. Transcriptomes of soft tissue tumors: pathologic and clinical implications. In: Expression Profiling of Human Tumors. M. Ladanyi, W.L. Gerald, eds. (2003) Humana Press, Totowa, New Jersey.

LETTERS TO THE EDITOR

1. O.W. Kamel, M. van de Rijn, R.A. Warnke, R. Dorfman. (1993) Reversible lymphomas. *New England Journal of Medicine* **329**:1658.

2. O.W. Kamel, M. van de Rijn, R.A. Warnke, R. Dorfman, G. Frizzera. (1995) On the origin of lymphoid neoplasms in rheumatoid arthritis. *Human Pathology* **26**:244.

3. M. van de Rijn, O.W. Kamel. (1995) Combination therapy for rheumatoid arthritis. *New England Journal of Medicine* **333**:1568.

4. M. van de Rijn, O.W. Kamel. (1996) Clonality of EBV in lymphoproliferative disorders in patients treated with immunosuppressive therapy for rheumatoid arthritis. *Journal of Clinical Oncology* **14**:3050-1.

5. A.B. Gelb, M. van de Rijn, R.A. Warnke, O.W. Kamel. (1997) Pregnancy associated lymphomas: a clinicopathological study. *Cancer* **79**:191-2.

6. K.E. Salhany, M. van de Rijn, K.T. Montone, J.E. Tomaszewski. (1997) CD5 immunostaining of lymphoid neoplasms in paraffin sections. *American Journal of Clinical Pathology* **107**:496.

ABSTRACTS

1. J. Hilkens, F. Buijs, J. Hilgers, Ph. Hageman, A. Sonnenberg, U. Kodovsky, K. Karande, R.P. van Hoeven, C. Feltkamp and M. van de Rijn. (1981) Monoclonal antibodies against human milk fat globule membranes detecting differentiation antigens of the mammary gland. *Protides of the Biological Fluids*, Vol. 29.

2. M. van de Rijn and I.L. Weissman. (1988) Biosynthesis of gpMel14, the murine lymph-node-specific homing receptor. In: The Ubiquitin System. Cold Spring Harbor. *Current Communications in Molecular Biology.*

3. D.L Kell, M. van de Rijn, J.J. Goates, O.W. Kamel, R.A. Warnke. (1993) Frozen section immunohistochemical analysis of cytokine receptor expression in B-lineage diffuse mixed small and large cell lymphomas. 5th International Conference on Human Leucocyte Differentiation Antigens. *Tissue Antigens* **42**:338.

4. V. Bhargava, H.N. Kirsch, R. Carlos, M. van de Rijn, O.W. Kamel, R.A. Warnke. (1994) Epstein-Barr virus associated T-cell lymphomas in Guatemala: an immunohistochemical and in situ hybridization study of seven cases. 83rd annual meeting, International Academy of Pathology, San Francisco.

5. V. Bhargava, M. Dolezal, M. van de Rijn, R.A. Warnke. (1994) Antigenic variability in non-Hodgkin's lymphomas accompanying changes in morphology: a study of 16 cases and their recurrences. 83rd annual meeting, International Academy of Pathology, San Francisco.

6. A. Gelb, M. van de Rijn, O.W. Kamel, R.A. Warnke. (1994) Pregnancy associated lymphomas: a clinicopathological study. 83rd annual meeting, International Academy of Pathology, San Francisco.

7. M. van de Rijn, C.M. Lombard, R.V. Rouse. (1994) Expression of CD34 by solitary fibrous tumors of the pleura, mediastinum, and lung. 83rd annual meeting, International Academy of Pathology, San Francisco.

8. D.A. Arber, O. W. Kamel, R. E. Davis, M. van de Rijn, L.M. Weiss. (1994) Detection of the Epstein-Barr virus in inflammatory pseudotumor. 83rd annual meeting, International Academy of Pathology, San Francisco.

9. O.W. Kamel, M.V. Dolezal, R.A. Warnke, M. van de Rijn. (1994) Clonal VDJ recombination by PCR in $CD20^+$ "usual" Hodgkin's disease. VIIth meeting, European Association for Haematopathology, Toledo, Spain.

10. M. van de Rijn, M.L. Cleary, R.A. Warnke, O.W. Kamel. (1995) Molecular features of Epstein-Barr virus-associated lymphoma in patients with rheumatoid arthritis. 84th annual meeting, International Academy of Pathology, Toronto.

11. M. van de Rijn, M. Hanasono, M. Billingham, O.W. Kamel. (1995) Is screening for EBV helpful in predicting the development of PTLD in cardiac transplant patients? Society for Cardiovascular Pathology, 10th anniversary meeting, Toronto.

12. O.W. Kamel, L.M. Weiss, M. van de Rijn, D.W. Kingma, E.S. Jaffe. (1996) Hodgkin's disease and Hodgkin's disease-like large cell lymphomas in patients receiving long-term low-dose methotrexate therapy. 85th annual meeting, International Academy of Pathology, Washington, D.C.

13. P. Chang, M. van de Rijn, R. Zeheb, R. Warnke, O. Kamel. (1996) Viral in situ hybridization studies in a clinical setting: comparison of manual methods with an automated in situ hybridization system. 85th annual meeting, International Academy of Pathology, Washington, D.C.

14. M. van de Rijn, O.W. Kamel, P.P. Chang, A.L. Lee, R.A. Warnke, and K.E. Salhany. (1996) Primary low grade B-cell lymphoma of the endometrial mucosa-associated lymphoid tissue. 85th annual meeting, International Academy of Pathology, Washington, D.C.

15. M. van de Rijn, K.E. Salhany, E. Davis, J. Hilkens, R.A. Warnke, O.W. Kamel. (1996) Reactivity of anti-CD56 antibody 123C3 on paraffin embedded tissues. 85th annual meeting, International Academy of Pathology, Washington, D.C.

16. M. van de Rijn, R.V. Rouse. (1996) Protein chemistry of CD34 expressed by solitary fibrous pleural tumors and gastrointestinal stromal tumors. 85th annual meeting, International Academy of Pathology, Washington, D.C.

17. C. Reynolds, M. van de Rijn, E.E. Furth, G.J. Berry, J.E. Tomaszewski, P. Belair. (1996) Myocyte apoptosis: a mechanism of cell death in cardiac allograft rejection? 85th annual meeting, International Academy of Pathology, Washington, D.C.

18. A.R. Judkins, K.T. Montone, V.A. Livolsi, M. van de Rijn. (1997) Sensitivity and specificity of antibodies on necrotic tumor tissue. 86th annual meeting, International Academy of Pathology, Orlando, FL.

19. M. van de Rijn, F.G. Barr, Q.B. Xiong, M. Hedges, J. Shipley, C. Fisher. (1998) Poorly differentiated synovial sarcoma: an analysis of clinical, pathologic amd molecular genetic features. Platform presentation, 86th annual meeting, International Academy of Pathology, Boston, MA.

20. M. van de Rijn, F.G. Barr, Q.B. Xiong, M. Hedges, C. Fisher. (1998) Specificity of t(x;18) for synovial sarcoma. 86th annual meeting, International Academy of Pathology, Boston, MA.

21. O.W. Kamel, E.A. Holly, M. van de Rijn, C. Lele, A. Sah. (1998) A population-based case-control study of non-Hodgkin's lymphoma in patients with rheumatoid arthritis. 86th annual meeting, International Academy of Pathology, Boston, MA.

22. J.M. Minda, M. van de Rijn, T.J. Lawton. (1998) Ultrastructural localization of CD34 in soft tissue tumors. Microscopy and Microanalysis, Atlanta, GA.

23. J. Goldsmith, M. van de Rijn, N. Syed. (1999) Orbital hemangiopericytoma and solitary fibrous tumor: lesions of similar pathogenesis? 87th annual meeting, International Academy of Pathology, San Francisco, CA.

24. M. van de Rijn, C. Perou, S. Jeffrey, M. Eisen, P. Brown, D. Botstein. (1999) Gene expression patterns in human breast cancer: correlation of microarray analysis with immunohistochemistry. Platform presentation, 87th annual meeting, International Academy of Pathology, San Francisco, CA.

25. J.R. Pollack, C.M. Perou, T. Sorlie, A.A. Alizadeh, C. Rees, M.B. Eise, A. Pergamenschikov, C.F. Williams, M. van de Rijn, S.S. Jeffrey, H. Johnsen, P.E. Lonning, S. Geisler, T. Aas, A-L. Borresen-Dale, D. Botstein, P.O. Brown. (1999) Genome-wide analysis of DNA copy number variation in breast cancer using DNA microarrays. *Nature Genetics* 23:3s-69.

26. T.O Nielsen, R. West, R. Tibshirani, M. Knowles, P. Brown, D. Botstein, M. van de Rijn. (2001) A molecular classification of soft tissue tumors using cDNA microarrays. (Stowell Orbison Award) 89th annual meeting, International Academy of Pathology, Atlanta, GA.

27. Y. Natkunam, R. Warnke, A. Alizadeh, R. Tibshirani, S. Horning, M. van de Rijn. (2001) bcl-6 expression predicts improved survival in patients with diffuse large B-cell lymphoma. Platform presentation, 89th annual meeting, International Academy of Pathology, Atlanta, GA.

28. B.T. Harris, K. Montgomery, M. van de Rijn. (2001) Tissue microarray analysis of normal and CNS/PNS structures and tumors. AANP Annual Meeting Abstract. *Journal of Neuropathology and Experimental Neurology* **60**(5):531.

29. J.P. Higgins, K. Montgomery, L. Wang, J.D. Brooks, M. van de Rijn. (2002) Expression of FKBP12 in benign and malignant vascular endothelium: an immunohistochemical study using conventional sections and tissue microarrays. 2002 annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

30. S.C. Linn, R.B. West, S. Zhu, T. Nielsen, J.R. Goldblum, R. Patel, B.P. Rubin, O. Alter, P.O. Brown, D. Botstein, M. van de Rijn. (2002) Genome-wide mRNA expression profiling of dermatofibrosarcoma protuberans using cDNA microarrays. 2002 annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

31. T.O. Nielsen, J.X O'Connell, F.D. Hsu, R.B. West, S.C. Linn, M. van de Rijn. (2002) Tissue microarray analysis of known and novel synovial sarcoma markers. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

32. R.B. West, S.C. Linn, E. Foxman, T. Neilson, S. Zhu, O. Alter, J.R. Goldblum, R. Patel, B.P. Rubin, P.O. Brown, D. Botstein, M. van de Rijn. (2002) The mRNA expression signature of solitary fibrous tumors. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

33. M. van de Rijn,, C. Perou, R. Tibshirani, O. Kallionemi, J. Kononen, G. Sauter, D. Botstein, P. Brown. (2002) Tissue microarray analysis of cytokeratin 17 and 5 expression shows association with poor clinical outcome in breast carcinoma. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

34. J.P.T. Higgins, R. Shinghai, J.H. Reese, M. Terris, R.J. Cohen, R. Tibshirani, G. Sherlock, M. Fero, J.R. Pollack, P.O. Brown, D. Botstein, M. van de Rijn, J.D. Brooks. (2002) Gene expression profiles in renal cell carcinoma assessed by complementary DNA microarray. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

35. R.B. West, S.C. Linn, T. Nielsen, S. Zhu, T. Longacre, A. Husain, O. Alter, R. Patel, P.O. Brown, D. Botstein, B.P. Rubin, J.R. Goldblum, M. van de Rijn. (2002) Distinction between low grade endometrial stromal sarcoma and smooth muscle tumors by cDNA gene array analysis. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

36. Z. Fan, M. van de Rijn, R. Rouse. (2002) Hep par-1 stain for the differential diagnosis of hepatocellular carcinoma: 613 tumors tested using tissue micro-arrays and conventional tissue sections. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

37. S.C. Linn, R.B. West, L.C. Wijnaendts, K. Montgomery, J. Mitchell, M. van de

Rijn. (2002) Tissue microarray analysis of CD44, p53, Ki-67 and bcl-2 as prognostic markers in rhabdomyosarcoma. 2002 Annual meeting United States and Canadian Academy of Pathology, Chicago, IL.

38. C.L. Lui, Y. Natkunam, W. Prapong, K. Montgomery, D. Botstein, P.O. Brown, M. van de Rijn. (2002) Software tools for high-throughput analysis and image retrieval of immunohistochemistry stains obtained on tissue microarrays. 2002 Annual meeting United States and Canadian Academy of Pathology. Chicago, IL.

39. L. Cheng, R.B. West, S. Zhu, S.C. Linn, T.O. Nielsen, J.R. Goldblum, R. Patel, B.P. Rubin, P. Brown, D. Botstein, M. van de Rijn. (2003) (Stowell Orbison Award) Expression profiling of fibromatosis by cDNA gene array analysis. 2003 Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

40. K. Jensen, R.B. West, S.X. Zhu, S.C. Linn, T.O. Nielsen, J.R. Goldblum, R. Patel, B.P. Rubin, D. Botstein, P. Brown, J. Pollack, B. Gilks, M. van de Rijn. Array-based comparative genomic hybridization (ACGH) of dermatofibrosarcoma protuberans (DFSP) on cDNA microarrays using DNA isolated from fresh frozen and paraffin embedded tissue. (2003) Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

41. R.B. West, S.C. Linn, T.O. Nielsen, K. Montgomery, J.R. Goldblum, R. Patel, B.P. Rubin, P. Brown, D. Botstein, M. van de Rijn. Novel gene FLJ10261 in gastrointestinal stromal tumors. (2003) Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

42. K. Jensen, M. Cheang, T.O. Nielsen, D. Huntsman, J. Ragaz, B. Gilks, M. van de Rijn, Tissue microarray analysis of cytokeratins 17 and 5/6 expression in breast cancer confirms association with poor outcome. (2003) Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

43. B. Gilks, V. Vanderhyden, S. Zhu, M. van de Rijn, T. Longacre. Distinction between serous borderline tumors and serous carcinomas based on mRNA expression profiling. (2003) Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

44. J.P. Higgins, L. Wang, N. Kambham, K. Montgomery, S. Vogelmann, K. Lemley, J.R. Pollack, M. van de Rijn, J.D. Brooks. Gene expression in the normal adult human kidney assessed by complementary DNA microarray. (2003) Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

45. A.E. Heerema, S. Linn, S. Zhu, M. van de Rijn. Rhabdomyosarcoma expresses genes associated with inhibition of myoblast differentiation. (2003) Annual meeting United States and Canadian Academy of Pathology. Washington, D.C.

46. A.E. Heerema, S.C. Linn, S. Zhu, M. van de Rijn. Clinical and biologic significance of MyoR mRNA expression in rhabdomyosarcoma, a tissue microarray study. (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

47. F.D. Hsu, M. Cheang, K. Jensen, M. van de Rijn, G. Karaca, C. Livasy, C.M. Perou, T.O. Nielsen. Epidermal growth factor receptor (HER1) is associated with basal-like breast cancers. (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

48. T. Morgan, M. van de Rijn, J. Higgins. Histidine decarboxylase expression is upregulated in the kidney of the pregnant mouse: implications for renal blood flow and hypervolemia of pregnancy. (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

49. M.A. Jorden, S. Zhu, M. van de Rijn. Gene expression profiling of distinct regions in normal autopsy brains: a study of three cases. (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

50. A. Alkushi, P. Lim, A. Coldman, D.G. Huntsman, D. Miller, A. Magliocco, M. van de Rijn, C.B.Gilks. Hierarchical clustering analysis of the immunostaining profiles of endometrial carcinomas identifies prognostically significant subsets of patients. Platform presentation, (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

51. R.B. West, C. Corless, M. Heinrich, S. Zhu, S. Subramanian, T.O. Nielsen, J.R. Goldblum, R. Patel, B.P. Rubin, P. Brown, M. van de Rijn. PDGFRa RNA Expression in neoplasia. Platform presentation, (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

52. S. Subramanian, R.B. West, S. Zhu, T.O. Nielsen, S.M. Dry, J.R. Goldblum, R. Patel, B.P. Rubin, P. Brown, M. van de Rijn. Extraskeletal myxoid chondrosarcoma: gene discovery using cDNA microarrays. Platform presentation, (2004) Annual meeting United States and Canadian Academy of Pathology. Vancouver, Canada.

SUBMITTED

1. C.B. Gilks, B.C. Vanderhyden, S. Zhu, M. van de Rijn, T.A. Longacre. Distinction between serous tumors of low malignant potential and serous carcinomas based on global mRNA expression profiling.

2. F.P. Li, J.A. Fletcher, M. Heinrich, J.E. Garber, S.E. Sallan, S. Higham, C. Curiel-Lewandrowski, A. Duensing, M. van de Rijn, L.E. Schnipper, G.D. Demetri. Familial gastrointestinal stromal tumor syndrome (GISTs): phenotypic and molecular features in a kindred.

IN PREPARATION

1. S. Subramanian, R.B. West, C.L. Corless, B.P. Rubin, K. Chu, S.Y. Leung, S.T. Yuen, S. Zhu, T. Hernandez-Boussard, K. Montgomery, T.O. Nielsen, J.R. Goldblum, R. Patel, M.C. Heinrich, J.A. Fletcher, M. van de Rijn. Gastrointestinal stromal tumors (GISTs) with KIT and PDGFRA mutations have distinct gene expression profiles