RESPRIDX EXECUTIVE SUMMARY

Patients with lung cancer are at increased risk for developing a second synchronous or metachronous lung tumor. The incidence of synchronous lung tumors has been variably reported between 1% and 16%. Metachronous lung tumors are likely more common, representing 40-60% of all patients with multiple lung tumors. Distinguishing whether a second tumor represents a *de novo* cancer or a recurrence/metastasis of the first cancer has important implications for treatment and prognosis. Specific clinical and pathological evidence is sought to resolve this issue.

De novo cancer formation of a second tumor is favored by a long latency interval (usually exceeding five years), better tumor differentiation, and occurrence in a site not typical of metastatic spread. Conversely, recurrent/metastatic formation of a second tumor is favored by a short interval to formation, similar histology with increased anaplasia, and multifocal tumor deposits. Immunohistochemistry (IHC) can be useful in differentiating *de novo* from recurrent/metastatic cancers, if unique staining differences between tumors can be demonstrated, such as when the second neoplasm is derived from a different cellular histogenesis (e.g. sarcoma with epithelioid growth characteristics *versus* a poorly differentiated carcinoma). However, when the second neoplasm is derived from the same cellular histogenesis (e.g. both tumors are squamous cell carcinomas or both tumors are adenocarcinomas), IHC provides limited value. Although the diagnostic yield of IHC is approximately 80%, uncertainty as to *de novo* or recurrent/metastatic disease remains in at least 20% of cases. Additionally, as cancers become more poorly differentiated, IHC profiles can also change leading to possible misdiagnosis of a new primary when actually representing a poorly differentiated form of the original well differentiated lesion.

Human malignancy arises, not from a single genomic alteration, but by a process of stochastic acquisition of cancer-related genomic damage over time. Clonal evolution involving individual cancer cells selected by greater expression of a malignant phenotype confers a unique fingerprint of genomic damage to each individual tumor. Recurrent and/or metastatic tumors share most, if not all, of the same genomic fingerprint patterns observed in the primary tumor from which they were derived. In contrast, *de novo* tumors manifest significantly different fingerprints of genomic damage.

RespriDX is a supplemental molecular pathology test that is useful in determining the *de novo* or recurrent/metastatic origin of disease when clinical and histopathology results are uncertain. Use of RespriDX to help stage the second lung tumor significantly impacts cancer treatment of the patient. RespriDX compares the genomic fingerprint of one lung tumor to that of a second synchronous or metachronous lung tumor using a robust panel of 25 DNA markers. The test can be performed on standard formalin fixed, paraffin embedded biopsy tissue or cytology slides resulting from fine needle aspirates, brushings or lavage preparations. RespriDX is based on two fundamental methodologies: i) pathologist-guided tissue microdissection at multiple sites of each tumor representative of the greatest cellular aggressiveness and ii) molecular genotyping for loss of heterozygosity (LOH) mutations associated with tumor suppressor genes and mutations in oncogenes. Genotyping results include the presence of each mutation on a specific allele, the clonality of that mutated allele, and the temporal sequence of such mutation acquisition through clonal expansion.

EVIDENCE FOR UTILITY OF RESPRIDX IN LUNG CANCER

Shen, C., et al. (2014). "Microsatellite alteration in multiple primary lung cancer." J Thorac Dis 6(10): 1499-1505. [1]

Patients with pulmonary neoplasms have an increased risk for developing a second tumor of the lung, either at the same time or different times. It is important to determine if the second tumor represents an independent primary tumor or recurrence/metastasis, because it will significantly change the management and prognosis. Microsatellite instability (MSI) and loss of heterozygosity (LOH) represents molecular disorders acquired by the cell during neoplastic transformation. Both are associated with genetic instability. Functional silencing of tumour suppressor genes may be the consequence of genomic instability, particularly of the globally occurring LOH phenomenon. Numerous studies have confirmed the role of MSI/LOH at both the early and the late stages of multiple primary lung cancer. This paper reviews the published literatures focused on the role of MSI/LOH significance in multiple primary lung cancer. Additionally, a new method based on the allelic variations at polymorphic microsatellite markers was offered that it does not rely on collection of normal tissue, performed with minimal tumor sample, and will complement clinical criteria for diagnostic discrimination between multiple primary cancers versus solitary metastatic diseases.

Saab, J., et al. (2017). "Utility of Genomic Analysis in Differentiating Synchronous and Metachronous Lung Adenocarcinomas from Primary Adenocarcinomas with Intrapulmonary Metastasis." Transl Oncol 10(3): 442-449. [2]

Distinguishing synchronous and metachronous primary lung adenocarcinomas from adenocarcinomas with intrapulmonary metastasis is essential for optimal patient management. In this study, multiple lung adenocarcinomas occurring in the same patient were evaluated using comprehensive histopathologic evaluation supplemented with molecular analysis. The cohort included 18 patients with a total of 52 lung adenocarcinomas. Eleven patients had a new diagnosis of multiple adenocarcinomas in the same lobe (n=5) or different lobe (n=6). Seven patients had a history of lung cancer and developed multiple new tumors. The final diagnosis was made in resection specimens (n=49), fine needle aspiration (n=2), and biopsy (n=1). Adenocarcinomas were non-mucinous, and histopathologic comparison of tumors was performed. All tumors save for one were subjected to ALK gene rearrangement testing and targeted Next Generation Sequencing (NGS). Using clinical, radiologic, and morphologic features, a confident conclusion favoring synchronous/metachronous or metastatic disease was made in 65% of patients. Cases that proved challenging included ones with more than three tumors showing overlapping growth patterns and lacking a predominant lepidic component. Genomic signatures unique to each tumor were helpful in determining the relationship of multiple carcinomas in 72% of patients. Collectively, morphologic and genomic data proved to be of greater value and achieved a conclusive diagnosis in 94% of patients. Assessment of the genomic profiles of multiple lung adenocarcinomas complements the histological findings, enabling a more comprehensive assessment of synchronous, metachronous, and metastatic lesions in most patients, thereby improving staging accuracy. Targeted NGS can identify genetic alterations with therapeutic implications.

Sasatomi, E., et al. (2002). "Comparison of accumulated allele loss between primary tumor and lymph node metastasis in stage II non-small cell lung carcinoma: implications for the timing of lymph node metastasis and prognostic value." Cancer Res 62(9): 2681-2689. [3]

Although the Tumor-Node-Metastasis staging of non-small cell lung carcinoma (NSCLC) is the most effective predictor of survival, the clinical outcome of patients at each stage is variable on an individual case basis. We tested the value of incorporating information about the tumor heterogeneity of NSCLC into microsatellite allelotyping in a cohort of 48 node-positive stage II patients (T1N1M0 and T2N1M0). Microsatellite allelotyping involved microdissection of the invasive component of primary tumor and lymph node metastasis at multiple target sites followed by loss of heterozygosity (LOH) analysis at specific regions on chromosomes 1p, 3p, 5q, 7q, 8q, 9p, 10q, 17p, and 18q using 16 markers. All microsatellites manifested LOH ranging from 44 to 76% in primary tumor and showed various degree of heterogeneity between primary tumor and lymph node metastasis. LOH on 3p and 5q in the lymph node metastases was associated significantly with shortened survival of the patients (P = 0.033 and 0.004, respectively), whereas no single LOH in the primary tumors showed association with prognosis. For the analysis of the accumulated load of allele loss, fractional allele loss (FAL) was calculated for each sample. The maximal FAL of lymph node metastasis was significantly lower than that of primary tumor (P = 0.0015), possibly reflecting the early lymphatic spread. High maximal FAL of lymph node metastasis was significantly correlated with an adverse outcome (P = 0.012), whereas maximal FAL of primary tumor did not show any prognostic significance (P = 0.552). A composite mutational profile for each patient based on the allelotyping of the primary tumor and lymph node deposits may make a significant contribution to a more accurate prognosis of stage II NSCLC.

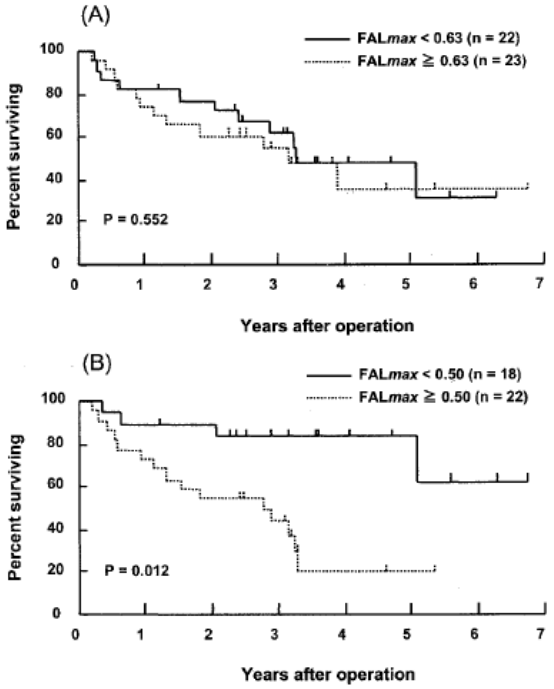

Fig. 4. Kaplan-Meier survival curves after curative resection of stage II NSCLC. Survival curves based on the FAL*max* of primary tumor (*A*). Survival curves based on the FAL*max* of lymph node metastasis (*B*).

those with a low FAL*max* (*P* _ 0.012).

- FAL (fractional allele loss) was defined as the ratio of chromosomes affected by LOH in the informative chromosomes and calculated for every microdissected sample. FAL*max* of the primary tumor and lymph node metastasis was determined separately in each case by selecting the largest FAL value from those of all microdissected samples in each component. FAL*min* was also calculated in the same manner.

- On the basis of the finding that the type and degree of chromosomal damage between primary tumor and lymph node metastasis were considerably different, the prognostic value of FAL*max* in primary tumor and lymph node metastasis was analyzed separately (Fig. 4). When patients were subclassified simply into two categories based on the median value of FAL*max,* FAL*max* in primary tumor did not show prognostic significance (*P* _ 0.552). On the other hand, patients whose metastatic deposits showed a high FAL*max* had a significantly greater risk of an adverse outcome than

Moffatt-Bruce, S. D., et al. (2010). "Comparative mutational profiling in the assessment of lung lesions: should it be the standard of care?" Ann Thorac Surg 90(2): 388-396.[4]

BACKGROUND: Discerning primary versus metastatic lung lesions is problematic. Comparative mutational profiling (CMP) involves genetic and point mutation analysis of lesions to facilitate this. We sought to review our experience in cases of two lung lesions or head and neck cancer and lung lesions to determine whether a significantly clinical problem existed, what standard processes were in place to address it, and whether a new diagnostic standard was required. METHODS: Between January 1, 2007, and October 31, 2008, CMP was used in 24 cases of two lung lesions or a head and neck cancer and lung lesion. Routine hematoxylin and eosin stain examination and immunohistochemistry were performed as appropriate. The CMP involved DNA sequencing for specific oncogene point mutations and a panel of allelic imbalance markers. Metastatic cancer required demonstration of concordant mutations affecting the same allele copy in different cancer deposits. RESULTS: The patient mean age was 62 years; there were 13 men and 11 women. The cases involved two lung lesions (n = 13) or a head and neck cancer and a lung lesion (n = 11). Standard pathology examination was unable to discriminate the lesions, and they were subsequently differentiated by CMP. Fifteen discordant CMP results were interpreted as independent primaries; 9 cases were concordant, consistent with metastatic disease. CONCLUSIONS: Discerning primary versus metastatic disease when dealing with lung lesions is a clinically significant problem. Comparative mutational profiling was found to be useful and reliable to assess the relatedness of multiple cancer lesions when routine pathology assessment was unable to.

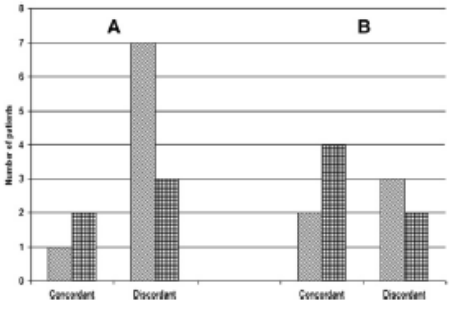

Fig 2. Synchronicity of lung lesions relative to concordance. Lesions that were detected within 6 months of each other were deemed synchronous (lighter hatched bars). (A) Among the patients with two lung lesions, there was a tendency, although not statistically significant, for the discordant lesions to occur synchronously. (B) Among the patients with a head and neck primary and a lung lesion, there was no significant difference in the timing of presentation of the lesion relative to the concordance of the lesion. (Darker hatched bars = metachronous).

- All the cases referred for *Comparative Mutational Profiling* (CMP) were determined to be metastatic (concordant mutations) or independent primary tumors (discordant mutations). Of the 24 cases referred for CMP, 15 were found to be discordant and thus representing two primary neoplastic processes. This could therefore be interpreted as a 63% discordance rate. Ten of the 14 discordant cases involved two separate lung lesions; five involved head and neck primaries separate from a lung primary (Fig 2). Although there was a tendency for the lung lesions to be discordant, this was not statistically significant (paired *t* test).

Table 3. Outcomes for Discordant Patients

Patient No.	Tumor Location	Surgical Therapy Offered	Outcomes	Follow-Up (Months)	Therapeutic Change
2	Lung/lung	Yes: declined	Alive chemotherapy, q3-month surveillance	30	No: patient declined and sought chemotherapy
4	Nasal/lung/lung	Yes: lobectomy	Alive, disease free, q3-month surveillance	25	Yes: completion lobectomy
5	Lung/lung/neck	No: no physiologic reserve	Alive, recurrence; treated with Tarceva	30	No: no physiologic reserve
6	Lung/lung	Yes: lobectomy	Alive, disease free	28	Yes: completion lobectomy
7	Lung/lung	No: pneumonectomy required	Alive, disease free	30	Yes: increased surveillance
8	Larynx/lung	Yes: lobectomy, but patient declined	Lost to follow-up	28	Yes: lobectomy offered but patient declined
11	Nasal/lung	Yes: lobectomy	Alive, disease free	25	Yes: completion lobectomy
12	Lung/lung	Yes: completion lobectomy	Alive, disease free	25	Yes: underwent completion lobectomy
13	Lung/lung/lung	No: no physiologic reserve	Alive, disease free	22	Yes; increase surveillance and chemotherapy
16	Lung/lung	Yes: lobectomy	Alive, treated with chemotherapy	28	Yes: completion lobectomy; cisplatin and navelbine and radiotherapy
18	Lung/lung	Yes: lobectomy	Alive, disease free	19	Yes: completion lobectomy
19	Lung/lung/lung	No: no physiologic reserve	Alive, disease free	20	No: no physiologic reserve
22	Lung/lung/lung	No: patient declined	Alive, disease free	15	Yes: increased surveillance
23	Lung/lung	Yes: lobectomy	Alive, adrenal metastases treated with chemotherapy	14	No: lobectomy was standard of care
24	Pharynx	No: patient declined	Alive, disease free	15	Yes: increase surveillance

- Surgical or additional therapy options were revisited for each of the patients with discordant lesions. Additional surgical intervention was offered in 9 of the 15 discordant cases (Table 3). The reasons for not offering further surgical intervention included lack of physiologic reserve (n _ 3), patient declined (n _ 2), and requirement for a pneumonectomy (n _ 1). All patients who underwent further surgical intervention tolerated their surgical procedures without complications and were discharged home. In 11 of the 15 discordant cases, the management of the patient was altered either in increased surveillance alone (n _ 3), additional surgical intervention (n _ 6), or chemotherapy administration (n _ 2). Recurrence developed in 2 patients: patient 5 at 12 months, and patient 17 at 8 months. Of note, patient 5 was stage IIIB at presentation. Patient 5 had a recurrence that was analyzed by CMP to verify that indeed the recurrence was a result of one of the original synchronous squamous cell lung cancers. The remainder of the patients remain alive and disease free, although some have had very short follow-up times (mean 24 months; range, 15 to 30; Table 3).

Girard, N., et al. (2009). "Genomic and mutational profiling to assess clonal relationships between multiple non-small cell lung cancers." Clin Cancer Res 15(16): 5184-5190. [5]

PURPOSE: In cases of multiple non-small cell lung cancer, clinicians must decide whether patients have independent tumors or metastases and tailor treatment accordingly. Decisions are currently made using the Martini and Melamed criteria, which are mostly based on tumor location and histologic type. New genomic tools could improve the ability to assess tumor clonality. EXPERIMENTAL DESIGN: We obtained fresh-frozen tumors specimens from patients who underwent surgery on at least two occasions for presumptively independent NSCLC. We did array comparative genomic hybridization (aCGH), mutational profiling of select genes, and detailed clinicopathologic review. RESULTS: We analyzed a total of 42 tumors from 20 patients (6 patients with synchronous tumors, 14 patients with metachronous tumors, 24 potential tumor pair comparisons); 22 tumor pairs were evaluable by aCGH.

Surprisingly, classification based on genomic profiling contradicted the clinicopathologic diagnosis in four (18%) of the comparisons, identifying independent primaries in one case diagnosed as metastasis and metastases in three cases diagnosed as independent primaries. Matching somatic point mutations were observed in these latter three cases. Another four tumor pairings were assigned an "equivocal" result based on aCGH; however, matching somatic point mutations were also found in these tumor pairs. None of the tumor pairs deemed independent primaries by aCGH harbored matching mutations. CONCLUSION: Genomic analysis can help distinguish clonal tumors from independent primaries. The development of rapid, inexpensive, and reliable molecular tools may allow for refinement of clinicopathologic criteria currently used in this setting.

- Tumors were analyzed by array comparative genomic hybridization (aCGH), a method that allows identification of copy number changes across the genome.

- aCGH was performed on all 42 tumor samples, but had to eliminate two cases from evaluation because of poor array quality. Thus, 22 tumor pairs were evaluable by aCGH. Overall, among the 22 paired comparisons, genomic profiling led to a diagnosis of metastases and multiple primaries in 4 and 14 cases, respectively. The conclusion was equivocal in the remaining 4 comparisons.

- Tumor mutation status (mutational profiling) is highly concordant with results from aCGH studies.

- The genomic classification contradicted the clinical diagnosis in 4 (18%) of 22 comparisons, identifying clonal patterns (metastases) in 3 (16%) cases diagnosed as independent primaries, and no evidence of clonality in 1 case diagnosed clinically as a metastasis. If the equivocal cases are considered to be a tentative diagnosis of metastases, then the genomic approach contradicted the clinical diagnosis in 7 of 22 comparisons (32%).

Dacic, S., et al. (2005). "Patterns of allelic loss of synchronous adenocarcinomas of the lung." Am J Surg Pathol 29(7): 897-902. [6]

Distinction of multiple primary lung carcinomas from intrapulmonary metastases using empiric clinical and histopathologic criteria can be difficult. Recent advances have provided several molecular markers that can be used for clonal analysis of separate tumor nodules and enhance tumor staging and subsequent treatment and prognosis. To address this issue, we performed a microdissection-based allelotyping of 20 cases of histologically similar, pathologic stage T4 adenocarcinomas (ADCs). Loss of heterozygosity (LOH) analysis included a panel of 15 polymorphic microsatellite markers located on 1p, 3p, 5q, 9p, 9q, 10q, 17p, and 22q. The tumor size, visceral pleural and angiolymphatic invasion, lymph node status, outcome, and survival were assessed. Allelotypes of 60 cases of solitary primary non-small cell lung carcinomas (NSCLC) (stages I-II) were used to define the percentage of discordant LOH patterns within solitary primary lung carcinoma that would discriminate between survivors and nonsurvivors. These criteria were used in the analysis of pathologic stage T4 ADC. Two groups of stage T4 cases were created: molecularly homogenous (< or = 40% discordances) (14 cases, 70%), and molecularly heterogenous (>40% discordances) (6 cases, 30%). Molecularly homogenous tumors were more frequently associated with visceral pleural invasion (92% vs. 8%) (P = 0.018). Allelotype did not correlate with age, gender, tumor size, tumor differentiation, lymph node status, angiolymphatic invasion, survival, or outcome. Our study showed that discordant and concordant genotypic profiles exist in morphologically similar synchronous ADC of the lung.

TABLE 2. Clinicopathologic Characteristics and Molecular Findings in 20 Synchronous Adenocarcinomas of the Lung

Case No.	Age (yr)	Sex	PL	AL	LN Status	TM Grade	Follow-Up	Outcome	Overall NI (%)	Informative Markers	Discordant LOH (%)
1	64	F	−	+	N2	PD	19	A	6.6	14	43
2	62	F	−	−	NX	MD	18	A	11	8	50
3	81	F	+	−	N0	MD	25	A	6.6	14	21
4	61	M	+	−	N0	MD	27	A	13.3	13	0
5	61	F	+	−	N0	WD	15	A	40	9	67
6	69	F	+	−	N0	MD	18	A	33	10	0
7	75	M	+	+	N1	PD	7	D	20	12	8.3
8	78	F	+	−	NX	MD	15	D	33	10	20
9	71	M	−	−	NX	MD	2	D	13.3	13	46
10	78	F	−	+	N2	MD	26	D	53	7	29
11	82	M	−	−	NX	MD	10	D	20	12	75
12	81	F	+	+	N1	MD	11	D	13.3	13	8
13	71	M	−	−	N0	MD	59	D	20	12	25
14	72	F	+	+	N1	PD	22	A	6.6	14	0
15	35	F	+	−	N2	PD	15	A	21	11	18
16	77	M	+	+	N0	PD	15	A	0	14	7.1
17	86	F	+	+	N0	MD	14	A	14	12	8
18	61	M	−	+	N0	MD	17	A	21	11	18
19	70	F	+	+	NX	MD	17	A	7	13	0
20	59	M	−	+	N0	PD	10	D	27	11	45

PL, pleural invasion; AL, angiolymphatic invasion; LN, lymph node; PD, poorly differentiated; MD, moderately differentiated; WD, well differentiated; A, alive; D, dead; NI, noninformative ratio; LOH, loss of heterozygosity.

- Of the 20 patients with synchronous ADC, 12 were women and 8 were men, with ages ranging from 35 to 86 years (mean, 70 years).

- Of the 15 patients (75%) with sampled lymph nodes, 6 patients (40%) had lymph node metastases. The main tumor mass ranged in size from 1.2 to 7.0 cm (mean, 2.5 cm). Complete follow-up until August 2004 or until death was obtained in all patients with a mean follow-up time of 18.1 months (range, 2–59 months). Eight patients (40%) died of disease.

- Based on the results of LOH analysis, two groups of synchronous ADCs were created: molecularly homogenous and molecularly heterogenous tumors.

- Lymph node metastases were present in 1 of 3 molecularly heterogenous cases (case nos. 1, 5, and 20, Tables 2 and 4), in which lymph node status was available. Although lymph node metastases were present in 5 of 12 molecularly homogenous cases, that difference was not significant (P = 0.659). Molecularly homogenous tumors were significantly more frequently associated with visceral pleural invasion (11 cases, 78.5%) than molecularly heterogenous cases (1 case, 16.7%; P = 0.018). Three patients (50%) from molecularly heterogenous group and 5 patients (35.7%) from molecularly homogenous group were dead at the end of this study. There was a tendency toward longer survival in the molecularly homogenous group of tumors (mean, 40 months; median, 59 months) than in the molecularly heterogenous group of tumors (mean, 13 months; median, 10 months), but that difference was not significant (P = 0.1588).

Dacic, S., et al. (2002). "Molecular pathogenesis of pulmonary carcinosarcoma as determined by microdissection-based allelotyping." Am J Surg Pathol 26(4): 510-516. [7]

Pulmonary carcinosarcoma is a rare, biphasic tumor composed of malignant epithelial and mesenchymal elements. Its histogenesis is controversial in light of the presence of divergent cell lineages and the clonal nature of malignancy. To address these issues, we performed an extensive comparative genotypic analysis using microdissection to secure representative mesenchymal and epithelial components from each of six cases of pulmonary carcinosarcoma. Loss of heterozygosity was analyzed with a panel of 12 polymorphic microsatellite markers designed to indicate allelic loss and situated in proximity to known tumor suppressor genes located on 1p, 3p, 5q, 9p, 10q, and 17p. In accordance with the relatively greater biologic aggressiveness of this tumor type, both the epithelial and mesenchymal components showed extensive allelic loss, most notably for 3p, 5q, and 17p. More importantly, we found overall equivalent patterns of acquired allelic loss between the two components on an individual case basis, strongly supporting the monoclonal origin of these neoplasms. Minor differences in the allelic fingerprint between the two cell lineages could be explained by progressive accumulation of allelic loss alterations that appear to occur more frequently in the mesenchymal component of the tumor. The data support the efficacy of microdissection-based allelic fingerprinting to delineate the relationship between different morphologic components of a single neoplasm.

TABLE 1. *Microdissection-based genotyping for LOH in primary carcinosarcomas of the lung*

		1p34 L-myc	3p26.1 OGG 1		5q21 APC/MCC		9p21 p16		10q23 PTEN		17p13 p53			
		5NT	D3S. 1539	D3S. 2303	D5S. 592	D5S. 615	D9S. 251	D9S. 254	D10S. 520	D10S. 1173	p53 I1	D17S. 974	D17S. 1303	FAL
1	Carcinoma	LOH	LOH	**No LOH**	LOH	LOH	NI	**No LOH**	NI	NI	LOH	NI	NI	0.8
	Sarcoma	LOH	LOH	**LOH**	LOH	LOH	NI	**LOH**	NI	NI	LOH	NI	NI	1.0
2	Carcinoma	LOH	LOH	NI	No LOH	LOH	**No LOH**	NI	LOH	LOH	NI	NI	LOH	0.83
	Sarcoma	LOH	LOH	NI	No LOH	LOH	**LOH**	NI	LOH	LOH	NI	NI	LOH	1.0
3	Carcinoma	No LOH	NI	NI	LOH	LOH	NA	NA	NA	NI	NI	**No LOH**	No LOH	0.33
	Sarcoma	No LOH	NI	NI	LOH +MSI	LOH	NA	LOH	No LOH	NI	NI	**LOH**	NA	0.67
4	Carcinoma	**No LOH**	LOH	NI	LOH	NI	NI	NI	NI	NI	LOH	LOH	LOH	0.75
	Sarcoma	**LOH**	LOH	NI	LOH	NI	NI	NI	NI	NI	LOH	LOH	LOH	1.0
5	Carcinoma	LOH	LOH	LOH	LOH	**LOH**	NI	LOH	LOH	NI	LOH	NI	LOH	1.0
	Sarcoma	LOH	LOH	LOH	LOH	**No LOH**	NI	LOH	LOH	NI	LOH	NI	LOH	1.0
6	Carcinoma	NA	NI	LOH	LOH	NI	NA	LOH	NI	LOH	NI	NI	LOH	1.0
	Sarcoma	NA	NI	LOH	LOH	NI	NA	LOH	NI	LOH	NI	NI	LOH	1.0

* The epithelial component was squamous cell carcinoma (case nos. 1–6). The mesenchymal component showed the following differentiation: myogenic (skeletal or smooth muscle; case nos. 1, 2, 4, and 5); cartilaginous and osteoid (case no. 3); rhabdomyosarcomatous and cartilaginous (case no. 6).

LOH, loss of heterozygosity; NI, non-informative; NA, not applicable; MSI, microsatellite instability; FAL, fractional allelic loss.

- The morphologic and genotypic findings of corresponding epithelial and mesenchymal component of each case are shown in Table 1. Informativeness for individual polymorphic microsatellite markers ranged from 25% to 100%. Both epithelial and mesenchymal tumor components showed extensive allelic loss with the highest frequencies of LOH seen on 3p26.1, 5q21, and 17p13 (Table 2).
- Statistical analysis demonstrated that fractional allelic losses at the chromosomal levels are more often present in mesenchymal than in epithelial component, suggesting accumulation of more advanced genetic changes in mesenchymal foci (p _0.035).

- Our study also suggests that carcinosarcomas of the lung evolve from the genetic transformation of a pure carcinoma into sarcoma. In addition, there appear to be subsets of pulmonary carcinosarcomas in which totipotent stem cell clone following genetic progression undergoes divergent differentiation into separate epithelial and mesenchymal directions.

Boute, P., et al. (2014). "Epidemiology, prognosis and treatment of simultaneous squamous cell carcinomas of the oral cavity and hypopharynx." Eur Ann Otorhinolaryngol Head Neck Dis 131(5): 283-287. [8]

OBJECTIVE: The study was designed to assess the prevalence, management and survival of patients with simultaneous squamous cell carcinomas of the oral cavity and hypopharynx (OC/HP). MATERIAL AND METHODS: A multicenter, retrospective study (2 university hospitals) was conducted between 2003 and 2007 on a series of 96 patients with simultaneous squamous cell cancers of the OC/HP. RESULTS: A total of 88 men and 8 women were included in the study: 81 patients presented double sites, 14 presented triple sites and one presented quadruple sites. The tumour sites most frequently observed were: hypopharynx in 61% of cases (involving the pyriform sinus in 42% of cases) and the oropharynx in 59% of cases (involving the palatine tonsil in 30% of cases). Upper aerodigestive tract endoscopy under general anaesthesia revealed a simultaneous lesion not suspected on clinical examination in 45% of patients: the site discovered on endoscopy was hypopharyngeal in 2 out of 3 cases; the tumour was classified T1 or T2 in 95.5% of cases. Patients treated simultaneously for all sites had a better prognosis than patients in whom each tumour was treated separately. The 5-year specific survival was 34% and the 5-year overall survival was 28%. CONCLUSION: The prevalence of simultaneous squamous cell carcinomas of the oral cavity and hypopharynx ranges between 1 to 7.4% in the literature and was 4.6% in the present series. A common treatment strategy for each of the patient's tumours appears to be superior to the current theoretical approach that consists of considering each tumour separately.

Cheng, H., et al. (2017). "Histologic lung cancer subtype differentiates synchronous multiple primary lung adenocarcinomas from intrapulmonary metastases." J Surg Res 211: 215-222. [9]

BACKGROUND: Distinguishing synchronous multiple primary lung cancers (SMPLCs) from intrapulmonary metastases is important. The objective of this study was to determine long-term survival in patients who underwent surgical resection for synchronous multiple lung cancers and identify additional criteria that may be useful to distinguish patients with SMPLCs from those with more advanced disease. METHODS: The medical records of patients with lung cancer who underwent planned resection for synchronous multiple lung cancers from 2007 to 2012 at our institutions were reviewed retrospectively. A comprehensive histologic assessment was used to determine whether the tumors were metastases or separate synchronous primary tumors. RESULTS: A total of 51 patients with synchronous multiple lung cancers underwent surgical resection. Twenty-nine patients had ipsilateral synchronous multiple lung cancers, and 22 had bilateral synchronous multiple lung cancers. No perioperative death occurred. The survival analysis of all 51 patients with synchronous multiple lung cancers who underwent planned resection of all lesions showed 3- and 5-year overall survival rates of 86% and 67%, respectively, The median overall survival was not reached. The comprehensive histologic assessment identified six patients with intrapulmonary metastasis and 45 patients with SMPLCs. Intrapulmonary metastases were associated with decreased survival. Among patients with SMPLCs, the epidermal growth factor receptor mutation distribution shown high concordant frequency rate of 35%

(5/14). CONCLUSIONS: Survival after surgical resection of synchronous multiple lung cancers in different lobes was promising. A comprehensive histologic assessment was useful for differentiating SMPLCs from intrapulmonary metastases.

Reck, M. and K. F. Rabe (2017). "Precision Diagnosis and Treatment for Advanced Non-Small-Cell Lung Cancer." N Engl J Med 377(9): 849-861. [10]

An individualized approach to the treatment of patients with NSCLC starts with an accurate pathological diagnosis and staging according to the eighth edition of the TNM classification for lung cancer70 and with the comprehensive use of appropriate imaging methods, as well as endoscopic techniques for tissue sampling. In addition to a precise description of histologic features, rational use of immunohistochemical markers is recommended. Patients with non–squamous-cell NSCLC should be screened for treatable oncogenic alterations, including *EGFR* mutations, *BRAF* V600E mutations, and *ALK* or *ROS1* translocations. Further molecular screening for rare treatable alterations is recommended in patients with adenocarcinoma who do not have a history of smoking. PD-L1 expression should be assessed in patients without known oncogenic alterations, regardless of the histologic findings (Figs. Figure 1 and Figure 2). A panel of appropriate specialists should oversee these evaluations to ensure that the diagnosis and staging are correct and that adequate tissue samples are obtained for molecular testing.

The choice of first-line treatment, based on the initial molecular pattern, includes chemotherapies, targeted therapies, and the new treatment option with pembrolizumab in patients with high levels of PD-L1 expression. Subsequent treatment options include chemotherapy combinations and immunotherapies in patients without oncogenic alterations, as well as targeted therapies for patients with refractory, molecular-driven tumors. Adequate tumor-biopsy samples obtained at the time of progression are crucial for the determination of the specific resistance mechanism19,20 (Figure 3and Table 1). The next step in precision diagnosis and treatment of lung cancer will be the identification of novel molecular markers, particularly those characterizing the likely response to immunotherapies.

EVIDENCE FOR UTILITY OF RESPRIDX IN OTHER CANCERS

Rolston, R., et al. (2001). "Distinguishing de novo second cancer formation from tumor recurrence: mutational fingerprinting by microdissection genotyping." J Mol Diagn 3(4): 129-132. [11]

PHS01-9594 JACOBS	1p MYCL	3p26 D3S 1539	3p26 D3S 2303	5q21 D5S 592	5q21 MCC	9p21 D9S 254	9p21 D9S 251	10q23 D10S 520	10q23 D10S 1173	10q23 MXI1	17p13 D17S 974	17p13 D17S 1289	17p13 D17S 1303
NORMAL COLON MUCOSA	I	I	I	I	NI	I	NI	I	I	I	I	I	I
COLON TUMOR 96-10688	LOH T	LOH B	LOH T	LOH B	NI	NO LOH	NI	LOH T	NO LOH	NO LOH	LOH B	NO LOH	NO LOH
LN MET 96-10688	LOH T	LOH T	LOH B	LOH B	NI	NO LOH	NI	LOH T	NO LOH	LOH T	LOH B	LOH T	LOH T
COLON TUMOR 01-3965	LOH T	LOH T	LOH B	LOH B	NI	NO LOH	NI	LOH T	LOH B	LOH T	LOH B	LOH T	LOH T

Figure 3. Microdissection genotyping. I: informative; NI: noninformative; NO LOH: no loss of heterozygosity (allelic balance); LOH: allelic loss. Initial allelic loss alteration is indicated in dark gray, second allelic loss event involving the same microsatellite is indicated in the four light gray boxes.

- Initially the colonic adenocarcinoma acquired the shared allelic loss alterations seen in both the primary colon cancer and its metastasis. Metastatic seeding occurred at that time leading to accumulation of new allelic loss alterations in the pericolonic lymph node metastasis. These new events in the pericolonic lymph node metastasis affected the same alleles subsequently altered in the primary colon cancer. The small intestinal tumor revealed 10 allelic loss alterations of which nine were not only identical with respect to the specific markers involved but also with respect to the specific alleles which had been lost (Figure 3). The fingerprint of the pericolonic lymph node metastasis and the subsequent small intestinal adenocarcinoma were very similar, differing only with respect to a single additional allelic loss alteration in the small bowel tumor. These genotypic features support the concept of a single colonic adenocarcinoma with small bowel metastasis after a five-year latency period.

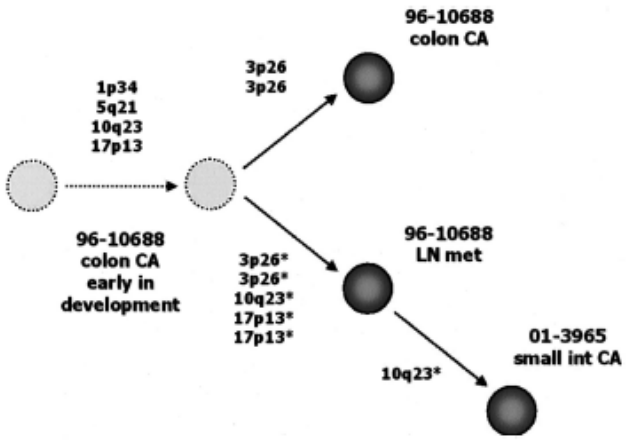

Figure 4. Temporal acquisition of mutational change. Initial shared allelic loss alterations are seen in both the primary colon cancer and lymph node metastasis. Metastatic seeding occurring at that time led to the accumulation of new allelic loss alterations in the lymph node metastasis which affected the same alleles subsequently altered in the primary colon cancer. The small intestinal tumor occurring five years later showed nine allelic loss alterations identical to that seen in the lymph node metastasis and one additional, new allelic loss.

- This case illustrates the practical difficulty of dealing with the presence of a second malignancy in a patient known to have had a previous cancer. Cogent arguments, based on clinical and histological criteria, may be made for the diagnosis of *de novo* tumor formation or for tumor recurrence/metastasis. Either of these could be contrary to that ultimately established by definitive mutational genotyping. Given the availability of techniques described in this report, the distinction can be performed in an objective manner using methods that are simple, high throughput, and cost effective.

Saad, R. S., et al. (2008). "Diagnostic and prognostic utility of molecular markers in synchronous bilateral breast carcinoma." Mod Pathol 21(10): 1200-1207. [12]

Histologic criteria have a limited role in determining whether the synchronous bilateral breast carcinomas represent two primaries or a metastasis to the contralateral breast. We studied the molecular analysis of synchronous bilateral breast carcinoma and whether they are originating from a single or different clone. We examined 17 patients with breast carcinoma, including 12 patients with synchronous bilateral carcinomas and control group of 5 infiltrating ductal carcinomas with regional lymph node metastases. Mutations were quantitatively determined to detect loss of heterozygosity (LOH) and microsatellite size alterations for a broad panel of 15 markers, involving 10 chromosomes using polymerase chain reaction. The carcinomas were classified as de novo or metastasis based on three levels of concordance: (1) marker-affected tumors were considered concordant if 50% or more of the same markers were mutated, (2) same gene copy affected, and (3) temporal sequence of mutation acquisition. In synchronous bilateral breast carcinoma patients, molecular analysis showed discordant mutations in all cases, supporting the diagnosis of de novo bilateral primary breast carcinomas. In patients with lymph node metastases, the primary breast carcinoma and metastases shared the same mutations, revealing a metastatic lesion. In conclusion, the application of molecular technology may play an important role for the differential diagnosis of dual primary carcinomas vs a metastatic breast cancer to contralateral breast. In this study, synchronous bilateral breast cancers represent two independent primaries rather than metastatic events.

Brinkmann, D., et al. (2004). "A molecular genetic and statistical approach for the diagnosis of dual-site cancers." J Natl Cancer Inst 96(19): 1441-1446. [13]

BACKGROUND: Concurrent tumors can be synchronous, independently derived, non-metastatic tumors or metastatic tumors. The prognosis and clinical management of patients with these different concurrent tumor types are different. METHODS: DNA from normal and tumor tissues of 62 patients with synchronous endometrial and ovarian, bilateral ovarian, or endometrial and bilateral ovarian tumors was analyzed for loss of heterozygosity and microsatellite instability using eight polymorphic microsatellite markers at loci frequently deleted in ovarian and/or endometrial cancers. A statistical algorithm was designed to assess the clonal relationship between the tumors. RESULTS: The original histopathology reports classified 26 (42%) case patients with single primary tumors and related metastatic lesions and 21 (34%) with independent primary tumors; 15 (24%) were unclassified. Genetic data identified 35 (56%) case patients with single primary tumors and related metastatic lesions, 18 (29%) with independent primary tumors, and nine (15%) that could not be typed. Excluding case patients with histopathology reports for which a clonal relationship was uncertain or was not reported, there was 53% concordance between genetic and histopathology diagnoses. Increasing the stringency of the statistical analysis increased the number of uncertain diagnoses but did not affect the proportion of discordant genetic and histologic diagnoses. CONCLUSIONS: We have developed a rapid and robust combined genetic and statistical method to establish whether multiple tumors from the same patient represent distinct primary tumors or whether they are clonally related and therefore metastatic. For the majority of case patients, histopathology reports and genetic analyses were in agreement and diagnostic confidence was improved. Importantly, in approximately one-fourth of all case patients, genetic and histopathologic analyses suggested alternative diagnoses. The results suggest that genetic analysis has implications for clinical management and can be performed rapidly as a diagnostic test with paraffin-embedded tissues.

Finkelstein, S. D., et al. (2003). "Microdissection-based allelotyping discriminates de novo tumor from intrahepatic spread in hepatocellular carcinoma." Hepatology 37(4): 871-879. [14]

A total of 103 cases of hepatocellular carcinoma (HCC) arising in native livers discovered at the time of transplantation underwent allelic loss analysis. HCC mutational allelotyping targeted 10 genomic loci (1p, 3p, 5q, 7q, 8q, 9p, 10q, 17p, 17q, 18q) using 18 polymorphic microsatellite markers situated in proximity to known tumor suppressor genes associated with human carcinogenesis. Gene analysis was performed on microdissected tissue samples removed from 4-microm thick histologic sections at specific topographic sites selected on the basis of representative cellular characteristics. Microdissection targets included largest tumor nodule at 2 locations as well as up to 3 additional tumor nodules in each case. HCC genotyping characteristics including mutational profile and cumulative fractional allelic loss (FAL) were correlated with clinical and pathologic features. Individual nodules of HCC showed 2 patterns of mutational change: (1) essentially concordant mutational profiles consistent with intrahepatic spread of tumor, or (2) discordant mutational profiles consistent with independent primary cancer formation. In 15 of 56 cases (27%) in which the HCC was in a multinodular, bilobar form (T4), sufficient discordance in the allelic loss profile enabled a more accurate T-stage classification with better prediction of recurrence-free survival. In conclusion, microdissection genotyping of HCC is an effective and objective means to (1) distinguish between de novo HCC tumor formation versus intrahepatic spread of cancer and to (2) improve on current methods for prediction of tumor aggressiveness and recurrence-free survival after liver transplantation.

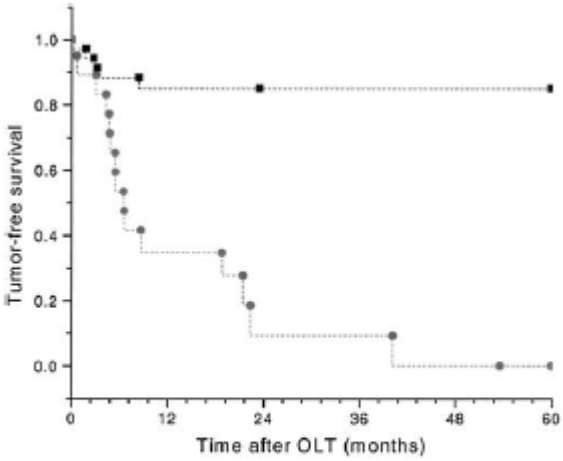

Fig. 5. Tumor-free survival stratified by 17p13-D17S-1289 ($P < .0001$). Tumor-free 5-year survival curves for patients with no allelic loss (0, ■) versus allelic loss (1, ●) is shown. Sixty-two of 103 (60%) patients in this series were found to be informative for this particular marker.

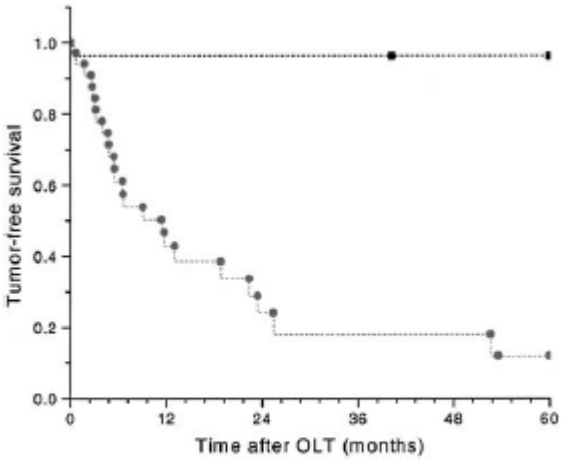

Fig. 6. Tumor-free survival stratified by 1p34-MYCL-5NT ($P < .0001$). Tumor-free 5-year survival curves for patients with no allelic loss (0, ■, n = 38) versus allelic loss (1, ●, n = 36) is shown. Seventy-four of 103 (72%) patients in this series were found to be informative for this particular marker.

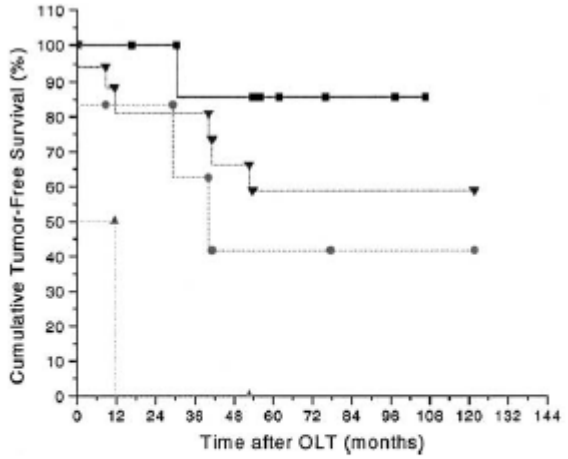

Fig. 8. Tumor-free survival for 18 patients of this series with bilobar HCC nodules originally classified as T4 using current TNM criteria. The overall survival of this cohort is shown by ▲. Based on microdissection-based genotyping it was possible to identify *de novo* cancer formation and reclassify the true significance of multinodular disease in each of these cases. The survival according to true T status is shown, in turn showing the efficacy of genotyping as a means to improve T classification of HCC. ■, Stages T1 and T2 (n = 9); ●, stage T3 (n = 6); △, stage T4 (n = 3); ▼, current TNM stage (n = 18).

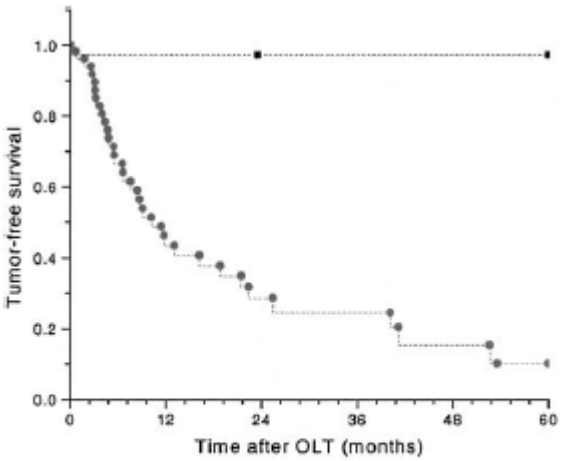

Fig. 7. Tumor-free survival stratified by FAL ($P < .0001$). Patients whose liver cancers displayed an FAL accumulated mutated fraction greater than 0.3 (●) showed a significantly lower 5-year tumor-free survival than those whose tumors manifested FAL indexes less than 0.3 (■).

Association Between FAL, Anatomic Tumor Characteristics, and Recurrence-Free Survival.
Nine of the 18 targeted microsatellites exhibited strong statistical association with recurrence-free survival. Two examples are shown in Figs. 5 and 6 corresponding to D17S1289 (17p13) and MYCL (1p36), respectively. However, because of the noninformative values for individual microsatellites, the total panel FAL was used for the statistical analysis. The FAL showed a significant association with recurrence-free survival (Fig. 7), vascular invasion, tumor number, tumor size, tumor differentiation, and lobar distribution (all *P* values_.0001). The multivariate analysis further identified only 3 of these variables as independent risk factors for recurrence: vascular invasion (Wald test *P*_.001), largest tumor size (*P* _ .003), and FAL (*P* _ .001).

Reclassification of T Stage Based on Molecular Analysis. Sixty patients in the study group had multiple tumors, 56 of which could be analyzed with respect to either *de novo* or *metastatic* based on genotyping. Of these, 18 patients had multiple bilobar tumors and, therefore, would have been classified automatically as T4 by the current tumor-node-metastasis (TNM) staging system. However, based on genotyping, these cases were reclassified as: T1 (N_1), T2 (N_8), T3 (N_6), or remained as T4 (N _ 3). As can be seen in Fig. 8, the recurrence free survival of these reclassified multinodular, bilobar tumor cases differed significantly from one another (*P* _ .02) and deteriorated with increasing T stage (as opposed to being encompassed within the same group according to the current TNM staging system).

Vollmer, R. T. (2009). "Primary lung cancer vs metastatic breast cancer: a probabilistic approach." Am J Clin Pathol 132(3): 391-395. [15]

In this study, a mathematical and probabilistic model is used to study the probability that a lung tumor is a primary vs a metastasis from cancer of the breast. The model uses information from immunohistochemical stains for thyroid transcription factor (TTF)-1, mammaglobin, p63, and estrogen receptor and epidemiologic data about primary lung and metastatic breast cancers in women. The

results demonstrate that these 4 stains can yield nearly certain diagnoses in approximately 80% of tumors falling into the pool of this differential diagnosis. Nevertheless, uncertainty of diagnosis remains for the 19% of tumors in the pool that are negative for TTF-1, mammaglobin, and p63.

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