Supplemental Materials

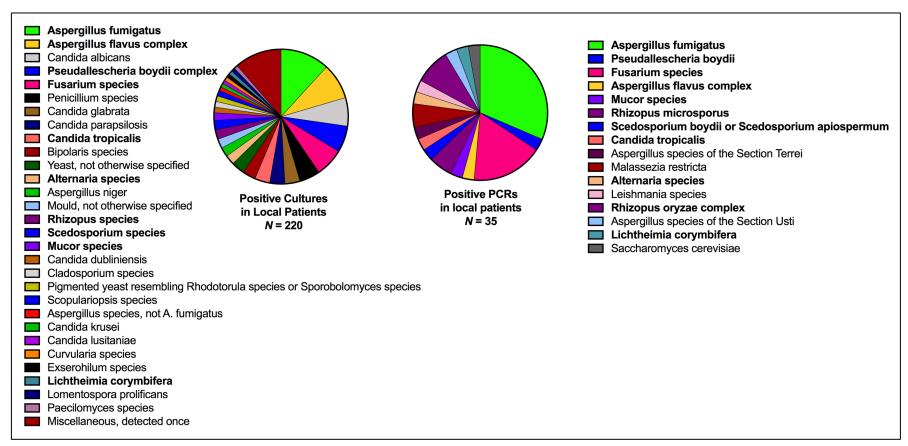
Supplemental Methods

Determination of Organism Prevalence

For all local patients with positive results, unique medical record-date combinations were determined and results by broad-range fungal PCR or fungal culture determined. For each organism identified the percent of total culture or PCR results, respectively, was calculated. For culture, the numbers of isolates for which sequence-based identification was performed and those isolates for which no identification was possible were recorded. Organisms identified only once were grouped into a single category.

Supplemental Results

There were 15 positive cultures from 15 unique patients identified with preliminary TATs longer than 300 hrs. Fourteen of these came from local patients, only three of whom were inpatients: two collecting in the operating rooms and one in the post-anesthesia care unit. An additional culture was collected in the pre-operative care unit, although this patient was designated an outpatient. None of these patients met inclusion criteria for chart review. The preliminary results from these outlier cultures comprised 6 unidentified organisms that required further incubation or sequence identification, two cultures that were positive for *Bipolaris spp*, and one culture each with *Exophiala spp*, *Exserohilum spp*, presumptive *Fusarium*, *Cladosporium spp*, *Pseudoallecheria boydii*, *Pleurostomophora richardsiae*, and one culture with both *Penicllium* and *Acremonium*. One mould could not be isolated from contaminating bacteria. Of the 9 cultures in which the number of colonies recovered was recorded, 7 had only one colony.



Supplemental Figure S1: Relative prevalence of fungal organisms detected by PCR and Culture from Local Patients.

The relative abundance of detected fungal organism by culture (left) and broad-range PCR (right) in all local patients, including those with chart review. Results were deduplicated to include only unique MRN-date combinations. Organisms detected by both methods are in bold with colors consistent across charts. Organisms detected only once by culture are grouped in a single category (N = 25 / 220 isolates). All *Fusarium spp* are grouped into a single category. Culture was unable to determine the identity of an organism for N = 3 isolates and sequence-based identification was required for N = 25 / 220 isolates. Our laboratory has detected *Saccharomyces cerevisiae* DNA by broad-range fungal PCR in multiple lots of Eswabs and report this finding with a disclaimer indicating a possible source of pre-analytical contamination. Parasites like *Leishmania spp* are occasionally detected by broad-range fungal PCR due to homology of the ribosomal gene within eukaryotes and are reported as an incidental finding.

Α

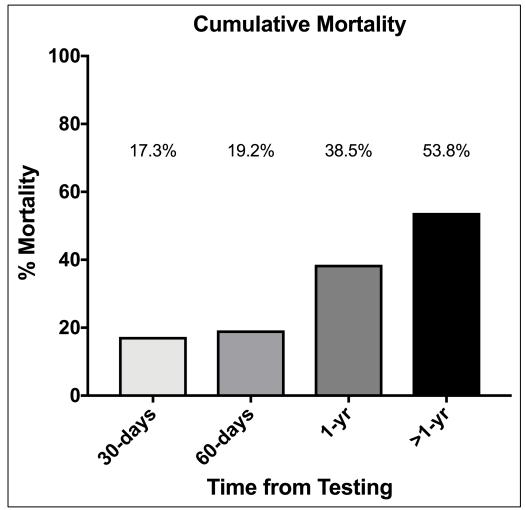
Sensitivity			Culture	Direct	Frozen	Both
	Culture	PCR	+ PCR	Stain	Section	Stains
Culture	-	0.1136	0.0269	0.0486	1.0	0.4136
PCR	0.1136	-	0.7370	0.0002	0.1375	0.5321
Culture + PCR	0.0269	0.7370	-	< 0.0001	0.0302	0.2972
Direct Stain	0.0486	0.0002	< 0.0001	-	0.0872	0.0068
Frozen Section	1.0	0.1375	0.0302	0.0872	-	0.5472
Both Stains	0.4136	0.5321	0.2972	0.0068	0.5472	-

В

D						
Specificity	Culture	PCR	Culture + PCR	Direct Stain	Frozen Section	Both Stains
Culture	-	1.0	1.0	0.4831	1.0	1.0
PCR	1.0	-	1.0	0.4831	1.0	1.0
Culture + PCR	1.0	1.0	-	0.2246	1.0	1.0
Direct Stain	0.4831	0.4831	0.2246	-	0.4000	0.4000
Frozen Section	1.0	0.1375	0.0302	0.4000	-	1.0
Both Stains	1.0	1.0	1.0	0.4000	1.0	-

Supplemental Figure S2. Statistical comparison of sensitivities and specificities for each fungal assay.

Results of Fisher's Exact test are reported as p-values for pairwise comparisons of **A**) sensitivities and **B**) specificities for fungal culture, broad-range fungal PCR, PCR and culture submitted in tandem, direct stain, frozen section, or direct stain and frozen section performed in tandem.



Supplemental Figure S3: Cumulative mortality for local patients with parallel fungal PCR and cultures.

Patient status as alive or dead at time of chart review and, if applicable, date of death was determined for the 52 patients included for chart review. Time to death from fungal testing was determined and binned as 0 - 30 days; 31 - 60 days; 61 - 365 days, or greater than 1 year and used to calculate cumulative mortality presented in the figure.

Supplemental Table S1: Total Specimens and Positivity Rates by Assay, Source Population, and Anatomic Site

Group	Unique	Culture	Culture	PCR	PCR	Cx vs PCR p-value ¹
	Patients	N (patients)	% pos	N (patients)	% pos	
All	1,459	1446 (949)	13.7%	644 (569)	37.1%	< 2.2e-16
Sinus						
Ref. lab	529	50 (31)	12.0%	543 (498)	35.9%	< 0.0005
Local	930	1396 (918)	13.8%	101 (71)	43.6%	3.73e-12
Patients						

¹Fisher's exact test.

Supplemental Table S2: Chart review data for all included patients with parallel fungal culture and broad-range PCR
Antifungal Direct Stain Frozen Section Post-Tes

			Antifungal			Direct Stain	Frozen Section	Post-Test	
Dations	tto de alcado e	Pre-Test	Exposure Prior			Results	Results	Assessment	Clinical
Patient	Underlying	EORTC	to Specimen	DCD DIt	Cultura Danult			of Fungal	Interpretation
Number	Diagnosis	Category	Collection	PCR Result	Culture Result			Disease	of Results
			fluconazole						
	Acute lymphocytic		prophylaxis;			No fungal	Hunhal alamants		
	leukemia, relapsed,		posaconazole			· ·	Hyphal elements,	Fungal	
1	s/p G-CLAM	Probable	treatment	Alternaria sp	NEG	elements	favor septate	etiology	True Positive
İ	Acute myelogenous		posaconazole	Fusarium			Tissue invasive		
	leukemia,		prophylaxis;	fujikuroi			fungal hyphae		
	refractory, s/p G-		amphotericin	species	Fusarium	2+ hyphal	and rare yeast	Fungal	
2	CLAM	Proven	В	complex	oxysporum	elements	forms	etiology	True Positive
			posaconazole;	Rhizopus					
	Diabetes mellitus,		amphotericin	oryzae	Rhizopus	Few hyphal	Suspicious for	Fungal	
3	type II	Proven	В	complex	<i>oryzae</i> complex	elements	fungal organisms	etiology	True Positive
	Large B-cell								
	lymphoma; history		oral				Atypical		
	of proven orbital		antifungals,			No fungal	lymphoid cell	Other	
4	aspergillosis	Possible	not specified	NEG	NEG	elements	proliferation	etiology	True Negative
	Diabetes mellitus,		amphotericin	Aspergillus	Aspergillus	Many hyphal		Fungal	
5	type II	Proven	В	fumigatus	fumigatus	elements	Not performed	etiology	True Positive
	71			, ,			, ,	Ů,	False Negative
	aplastic anemia, s/p								(degraded
	matched related						Inflamed		hyphae seen on
	bone marrow		isavuconazole,			No fungal	granulation	Fungal	final
6	transplant	Probable	voriconazole	NEG	NEG	elements	tissue	etiology	histopathology)
	aplastic anemia, s/p		amphotericin	Aspergillus		moderate	Tissue invasive	Fungal	
6	matched related	Proven	В	fumigatus	NEG	hyphae	fungal hyphae	etiology	True Positive

	bone marrow								
	transplant								
	aplastic anemia,								
	chemotherapy prior						Negative for		
	to matched related						Negative for		
	bone marrow					No fungal	invasive fungal	Fungal	
6	transplant	Probable	voriconazole	NEG	NEG	elements	elements	etiology	False Negative
	Acute myelgenous		isavuconazole,						
	leukemia, relapsed,		amphotericin	Fusarium	Presumptive	No fungal	Positive for	Fungal	
7	s/p CLAM	Probable	В	solani	Fusarium solani	elements	fungal elements	etiology	True Positive
	chronic						Consol books		
	myelogenous						Fungal hyphae		
	leukemia with blast		isavuconazole	Lichtheimia	Lichtheimia	Many hyphal	with necrotic	Fungal	
8	crisis	Proven	prophylaxis	corymbifera	corymbifera	elements	debris	etiology	True Positive
			micafungin,						
	Acute myelgenous		amphotericin,						
	leukemia, relapsed,		isavuconazole	Fusarium	Fusarium	Many hyphal	No definite	Fungal	
9	s/p G-CLA	Proven	prophylaxis	fujikuroi	fujikuroi	elements	fungal elements	etiology	True Positive
							No fungal		
	Chronic lymphocytic		amphotericin			No fungal	elements	Other	
10	leukemia	Possible	В	NEG	NEG	elements	identified	etiology	True Negative
	myelofibrosis, s/p			Aspergillus		No fungal		Fungal	
11	PBSCT	Probable	voriconazole	fumigatus	NEG	elements	Fungal hyphae	etiology	True Positive
11	Acute lymphocytic	FIODADIE	VOLICOLIAZOIE	Rhizopus	INLG	elements	Tungarnyphae	etiology	True Positive
	leukemia, s/p non-			oryzae		No fungal	Positive for	Fungal	
12	myeloablative HSCT	Proven	none	complex	Rhizopus sp.	elements	fungal elements	etiology	True Positive
12	Thyeloablative HSC1	Pioveii	none	complex	Candida	elements	Tungar elements	etiology	True Positive
	History of afrin &				tropicalis,	Moderate	Suspicious for		
	intranasal	Not		Candida	Scopulariopsis	fungal	invasive fungal	Other	Bystander/colo
13	acetominophen	Applicable	none	tropicalis	brevicaulis	elements	hyphae	etiology	nizer
13	Sinonasal squamous	Аррисавіе	none	tropicalis	Dievicuuis	elements	Пурпас	etiology	IIIZEI
	cell carcinoma, s/p								
	chemotherapy &			Aspergillus	Aspergillus	No fungal		Fungal	
1.4		Drovon	flucopazolo	fumigatus		elements	Not performed	_	True Positive
14	radiotherapy	Proven	fluconazole	juinigutus	fumigatus	CICILICITIS	TVOL PELJOITHEU	etiology	True Positive
	Acute myelogenous leukemia, s/p G-		fluconazole, amphotericin			No fungal		Other	
15	CLAM	Possible	B B	NEG	NEG	elements	Not performed		True Negative
13	CLAIVI	LOSSIDIG	D		INEU	elements	ivot perjornieu	etiology	True Negative
	Acute myelogenous		amphatarisin	Aspergillus sp	Asporailles	No fungal		Fungal	
16	, ,	Droboble	amphotericin	of Section	Aspergillus	_	Not parformed	Fungal	True Desitive
16	leukemia, s/p PBSCT	Probable	B, micafungin	Terrei	terreus	elements	Not performed	etiology	True Positive
	Indus abdentinal				Scedosporium	No fungal	Negative for	Fun and	
17	Intra-abdominal	Dunhalala		NEC	apiospermum	_	_	Fungal	Truce Decition
17	sepsis; History of	Probable	none	NEG	complex	elements	fungal elements	etiology	True Positive

	liver and kidney transplant								
	Acute myelogenous			Aspergillus			Positive, appear		
	leukemia, receiving			flavus		No fungal	[to be] septate	Fungal	
18	chemotherapy	Proven	none	complex	NEG	elements	hyphae	etiology	True Positive
	Acute myelogenous			Fusarium		Many fungal	Positive for	Fungal	
19	leukemia	Proven	posaconazole	solani	Fusarium solani	elements	fungal forms	etiology	True Positive
	Acute myelogenous						Ni a a a l'a l'a a a		
	leukemia, s/p MEC;						Necrotic tissue		
	concurrent A.						with fungus.		
	fumigatus						Viable tissue		
	pneumonia		Isavuconazole,	Aspergillus	Aspergillus	Many fungal	suspicious for	Fungal	
20	(antecedent dx)	Proven	micafungin	fumigatus	fumigatus	elements	fungal forms.	etiology	True Positive
	Acute lymphocytic		fluconazole						
	leukemia, s/p		prophylaxis;						
	rituximab and		amphotericin			Many fungal	Numerous fungal	Fungal	
21	cytarabine	Proven	В	Mucor sp	Mucor sp	elements	hyphae	etiology	True Positive
	Myelodysplastic					Occasional			
	syndrome, s/p		amphotericin	Aspergillus	Aspergillus	fungal	Negative for	Fungal	
22	guadecitabine	Probable	В	fumigatus	fumigatus	elements	fungal elements	etiology	True Positive
							Positive for		
	Acute myelogenous		voriconazole,				fungal organisms,		
	leukemia, s/p G-		amphotericin	Fusarium		Many fungal	appear	Fungal	
23	CLAM	Proven	В	fujikuroi	Fusarium sp	elements	angioinvasive	etiology	True Positive
23	Chronic lymphocytic	TTOVEIT	В	jujikuroi	r usurium sp	Cicincitis	angionivasive	ctiology	True rositive
	leukemia, s/p CAR-T					No fungal		Fungal	
24	cell infusion	Probable	none	A. fumigatus	NEG	elements	Not performed	etiology	True Positive
	- Centinusion	11000010	voriconazole,	Rhizopus	1120		Tree perjetities	ctiology	True rositive
	Aplastic anemia s/p		micafungin,	microsporus					
	haploidentical bone		amphotericin	or <i>Rhizopus</i>		No fungal		Fungal	
25	marrow transplant	Probable	В	azygosporus	Rhizopus sp	elements	Not performed	etiology	True Positive
-	Acute myelogenous			/ 5 - 4 - 5 - 5 - 5	-1		, , , , , , , , , , , , , , , , , , ,		
	leukemia, multiple		Amphotericin	Rhizopus		No fungal	No definite	Fungal	
26	myeloma	Probable	В	oryzae	NEG	elements	fungal organisms	etiology	True Positive
	Acute myelogenous		Amphotericin	Mucor		Many fungal		Fungal	
27	leukemia	Proven	В	circinelloides	Mucor sp	elements	Not performed	etiology	True Positive
			posaconazole,			2.3		- 3.0.061	
	Acute myelogenous		amphotericin			No fungal	No fungal forms	Other	
28	leukemia	Possible	В	NEG	NEG	elements	identified	etiology	True Negative
	Acute myelogenous					No fungal		Other	
29	leukemia, type 1	Possible	posaconazole	NEG	NEG	elements	Not performed	etiology	True Negative
23	icakciiia, type i	1 0331010	Posaconazoie	1110	1120	Cicinciita	. voc perjornica	chology	True Negative

Acute lymphocytic leukemia, s/p CAR-T infusion Acute myelogenous amphotericin B										
Acute myelogenous leukemia, relapsed s/p FLAG Acute lymphocytic leukemia, s/p CAR-T 31 Infusion Possible micafungin NEG NEG elements Acute myelogenous leukemia, s/p CAR-T Acute myelogenous leukemia, relapsed Probable B Acute myelogenous leukemia, s/p CAR-T Acute myelogenous leukemia, s/p cord blood transplant Acute myelogenous	[DM, IVDU,								
Acute myelogenous elements Fungal hyphae presents; at least Fungal hyphae presents Fungal hyphae presents Fungal hyphae presents Fungal hyphae presents Fungal hyphae Proven Proven hyphae Proven Proven hyphae Proven hyph	6	endocarditis								
Acute lymphocytic leukemia, s/p CAR-T infusion Acute myelogenous leukemia, relapsed Acute myelogenous leukemia, s/p Acute myelogenous leukemia, s/p Acute lymphoctic myelogenous leukemia, s/p Acute lymphoctic leukemia, s/p Acute lymphoctic leukemia, s/p Acute lymphoctic leukemia possible Acute lymphoctic leukemia possible Acute lymphoctic leukemia /p cord blood transplant Acute lymphoctic leukemia Acute myelogenous leukemia posaconazole prophylaxis amphotericin Acute myelogenous leukemia posaconazole prophylaxis, amphotericin Acute myelogenous leukemia, evolved Acute lymphoma with HLH Proven Aspergillus Aspergillus Aspergillus Aspergillus Aspergillus Aspergillus Aspergillus Appergillus App	I	leukemia, relapsed		•	microsporus		•	present; at least	_	
Ieukemia, s/p CAR-T Infusion Possible micafungin NEG NEG elements fungal forms etiology True			Probable	В	azygosporus	Rhizopus sp	elements	focally invasive	etiology	True Positive
Acute myelogenous leukemia, relapsed Proven B amphotericin B boydii or S. apiospermum boydii complex elements seen Fungal etiology True I elements seen elements seen True I apiospermum boydii complex elements seen Fungal etiology True I apiospermum boydii complex boydii complex elements seen Fungal etiology True I apiospermum boydii complex boydii complex boydii complex boydii complex possible fragility apiospermum boydii complex possible seen Fungal etiology True I apiospermum boydii complex possible fragility for culture and PCR culture and PCR concordance. Possible prophylaxis plaus positive result for culture and PCR concordance. Positive for fungal forms with elements angioinvasion etiology True I for fungal forms with angioinvasion etiology True I posaconazole prophylaxis prophylaxis, amphotericin posaconazole prophylaxis, amphotericin amphotericin posaconazole prophylaxis, amphotericin amphotericin amphotericin posaconazole prophylaxis, amphotericin amphoteri	I	leukemia, s/p CAR-T	Possible	micafungin	NEG	NEG	_			True Negative
Acute myclogenous leukemia, s/p Possible Possible B Rare hyphae reported; determined to be diagnostic error; treated as positive result for culture and PCR Other amphotericin posaconazole, leukemia s/p cord blood transplant Possible Prophylaxis NEG NEG elements Concordance. etiology True I Possible Prophylaxis NEG NEG elements Possible prophylaxis NEG NEG elements Possible Prophylaxis NEG NEG elements No fungal elements I Rare possible fungal fynamic elements No fungal elements I Rare possible fungal hyphae; no tissue, mucus only; bacterial of the prophylaxis, amphotericin Neg Neg elements forms etiology True I No fungal elements forms etiology True I No fungal elements forms Possible Prophylaxis amphotericin Neg Neg elements forms etiology True I No fungal elements forms Possible Prophylaxis amphotericin Neg Neg elements forms etiology True I No fungal elements forms Possible Prophylaxis amphotericin Neg Neg elements forms Possible Prophylaxis amphotericin Neg Neg elements forms Possible Prophylaxis Probable Prophylaxis amphotericin Neg Neg elements forms Possible Prophylaxis amphotericin Neg Neg elements forms Possible Prophylaxis elements forms Possible Prophylaxis elements fungal hyphae elements fungal infection etiology True I Possible Prophylaxis allescheria boydii boydii boydii complex elements fungal infection etiology True I Possible Prophylaxis allescheria boydii poydii poydii poydii poydii poydii elements fungal infection etiology True I Possible Prophylaxis poydii poydii poydii poydii elements fungal infection etiology True I Possible Prophylaxis poydii poydi			Proven	•	boydii or S.	allecheria	_	fungal hyphae	_	True Positive
Ieukemia, s/p matched PBSCT Possible prophylaxis NEG NEG elements culture and PCR concordance. etiology True I	,	Acute			, ,	, ,		reported; determined to be diagnostic error; treated as	3,	
matched PBSCT Possible prophylaxis NEG NEG elements concordance. etiology True I Acute lymphoctic amphotericin Acute lymphoctic leukemia, s/p cord blood transplant Possible prophylaxis NEG NEG NEG elements angioinvasion etiology True I Acute myelogenous leukemia, evolved Acute myelofibrosis Possible B NEG NEG NEG NEG NEG elements from myelofibrosis Possible B NEG NEG elements No fungal elements from Sisue, mucus only; bacterial Other etiology True I Aspergillus Aspergillus No fungal elements Not performed etiology True I Rare possible fungal hyphae; no tissue, mucus only; bacterial Other etiology True I Aspergillus Aspergillus fungalus fumigatus fumigatus elements forms etiology True I Moderate hyphae elements fungal hyphae etiology True I Moderate fungal No evidence of Fungal elements boydii complex elements fungal infection etiology True I Positive for fungal fungal elements etiology True II No fungal elements fungal infection etiology True II No fungal elements fungal infection etiology True II No fungal elements fungal infection etiology True II No evidence of fungal infection etiolo		. •		voriconazole			No fungal	culture and PCR	Other	
Acute lymphoctic leukemia Possible B flavus flavus Possible B NeG NeG elements Possible fungal forms with angioinvasion etiology True I Possible prophylaxis No fungal elements Possible fungal hyphae; no tissue, mucus only; bacterial lymphoma with HLH Proven none funigatus prophylaxis, allescheria prophylaxis, capsofungin Possible probable prophylaxis, allescheria boydii complex elements fungal hyphae elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology True I Possible boydii complex elements fungal infection etiology I True I Possible boydii complex elements fungal infection etiology I True I Possible boydii complex elements fungal infection etiology I True I Possible I Possible boydii complex elements fungal infection etiology I I I I I I I I I I I I I I I I I I I		-	Possible	prophylaxis	NEG	NEG	elements	concordance.	etiology	True Negative
leukemia, s/p cord blood transplant Possible prophylaxis NEG NEG elements No fungal elements Not performed etiology True II Rare possible fungal hyphae; no tissue, mucus only; bacterial only; bacterial forms etiology True II Acute myelogenous leukemia, evolved amphotericin B NEG NEG elements forms etiology True II Cutaneous T-cell S Iymphoma with HLH Proven none fumigatus fumigatus elements fungal hyphae etiology True II No fungal only; bacterial only; bacterial forms etiology True II Aspergillus fumigatus elements fungal hyphae etiology True II No evidence of Fungal S Iymphoma with HLH Proven none fumigatus allescheria allescheria fungal boydii boydii omplex elements fungal infection etiology True II No evidence of Fungal S Iymphoma infection etiology True II Pencillium sp		• •	Possible	amphotericin			_	fungal forms with	_	True Positive
Acute myelogenous leukemia, evolved 36 from myelofibrosis Possible B NEG NEG elements forms etiology True I Cutaneous T-cell lymphoma with HLH Proven none fumigatus fumigatus elements fungal hyphae etiology True I No fungal only; bacterial forms etiology True I Aspergillus Aspergillus hyphae elements fungal hyphae etiology True I Voriconazole prophylaxis, allescheria allescheria allescheria fungal No evidence of Fungal elements fungal infection etiology True II No evidence of Fungal elements fungal infection etiology True II Pencillium sp	1	leukemia, s/p cord	Possible	•	NEG	NEG	_	Not performed		True Negative
Cutaneous T-cell Proven none Aspergillus fumigatus fumigatus fumigatus fumigatus fumigatus fumigatus fumigatus fungal hyphae elements fungal hyphae etiology True Fingal etiology True Fingal No evidence of Fungal Pseud- allescheria fungal No evidence of Fungal etiology True Fingal fungal infection etiology True Fingal fungal infection etiology True Fingal fungal infection fungal infection etiology True Fingal fungal infection etiology fungal infection fungal inf	1	leukemia, evolved	Possible	prophylaxis, amphotericin	NEG	NEG	_	fungal hyphae; no tissue, mucus only; bacterial		True Negative
voriconazole prophylaxis, allescheria allescheria boydii complex elements fungal infection etiology True F							hyphae	fungal humbaa	Fungal	T 0 111
prophylaxis, allescheria allescheria fungal No evidence of Fungal elements Myelofibrosis Probable capsofungin boydii boydii complex elements fungal infection etiology True Fungal elements	37 l	lymphoma with HLH	Proven	none	fumigatus	fumigatus		rungai nyphae	etiology	True Positive
	38 1	Myelofibrosis	Probable	prophylaxis,	allescheria	allescheria boydii complex	fungal			True Positive
	39 I	HIV (on HAART)		None	NFDD	AND Cephalotheca	2+ Yeast seen	Not performed		Bystander/colo

	T	1	ı	1	1	T	T	1	1
					Phialemonium				
					inflatum				
	Acute lymphocytic						Tiesus impresive		
	leukemia, salvage						Tissue invasive		
	chemotherapy;						fungus present;		
	concurrent fungal						"size of		
	pneumonia (A.					Moderate	organisms		
	fumigatus, Fusarium					fungal	suggests	Fungal	
40	_	Proven	nasasanazala	Fusarium en	Fusarium sn	elements	zygomycetes"	_	True Positive
40	spp)	Proven	posaconazole	Fusarium sp	Fusarium sp	elefficits	zygomycetes	etiology	True Positive
	Diabetes mellitus								
	type II, distant								
	history of								
	chraniopharyngioma	Not				No fungal		Other	
41	and CSF leak	Applicable	None	NEG	NEG	elements	Not performed	etiology	True Negative
	Acute myelogenous								
	leukemia, relapsed;		voriconazole						
	history of sinus		prophylaxis,			No fungal	No definite	Other	
42	aspergillosis	Possible	isavuconazole	NEG	NEG	elements	fungal organisms	etiology	True Negative
	Acute myelogenous								_
	leukemia (secondary						No definitive		
	to DLBCL						fungal forms;		
	treatment), sp		amphotericin		Aspergillus	No fungal	recent prior	Fungal	
43	chemotherapy	Possible	В	NEG	fumigatus	elements	positive result	etiology	True Positive
1.5	one merapy	1 0331010		1120	Aspergillus	Cicincino	positive result	ctiology	True rositive
				Aspergillus	ustus complex				
	Myolodycoloctic			ustus and	and	Moderate			
	Myelodysplastic					hyphal		Fa.aal	
	syndrome, s/p			Scopulariopsis	Scopulariopsis		Matural and a market	Fungal	
44	PBSCT	Possible	voriconazole	brevicaulis	brevicaulis	elements	Not performed	etiology	True Positive
	Liver transplant,								
	plasma cell			Scedosporium	_				
	hepatitis,			<i>boydii</i> or	Pseud-	No. Co.			
	concurrent SSTI with			Scedosporium	allescheria	No fungal		Fungal	
45	Scedosporium spp	Probable	voriconazole	apiospermum	<i>boydii</i> complex	elements	Not performed	etiology	True Positive
	Acute lymphocytic								
	leukemia, s/p CAR-T								
	infusion; fungal								
	elements on								
	histopathology (PCR								
	on FFPE positive for								
	A. fumigatus								
	months later, no		amphotericin						
	paired culture with		В,			No fungal		Fungal	
46	that specimen)	Proven	posaconazole	NEG	NEG	elements	Not performed	etiology	False Negative
40	that specimen)	FIUVEII	posaconazole	INLU	INLG	Cicilients	TVOL PELJOITHEU	enology	i aise ivegative

									1
	Acute lymphocytic leukemia, s/p		fluconazole prophylaxis, amphotericin	Aspergillus	Aspergillus	Few hyphal		Fungal	
47	chemotherapy	Proven	В	fumigatus	fumigatus	elements	Not performed	etiology	True Positive
	Acute myelogenous		posaconazole	Aspergillus		No fungal		Fungal	
48	leukemia, secondary	Possible	prophylaxis	fumigatus	NEG	elements	Not performed	etiology	True Positive
							Not performed;		
							recent prior		
	B-cell lymphoma					No fungal	positive for		
	(Imited specimens					elements;	septate hyphae		
	for both culture &					only swab	in decalcified	Other	
49	PCR)	Proven	none	NEG	NEG	submitted	specimen	etiology	False Negative
	Intracranial abscess,								
	meningitis, and								
	bacteremia due to								
	Staphylococcus	N1 - 4		0.4-1		No fungal	No apparent	Other	Durata a da a/a ala
F0	aureus with sinus	Not		Malassezia	NEC		1 ' '	Other	Bystander/colo
50	involvement	Applicable	none	restricta	NEG	elements	fungal organisms	etiology	nizer
							No obvious		
	EtOH abuse and						fungal forms or		
	ESRD with marked			Rhizopus		No fungal	significant	Fungal	
51	anemia	Proven	none	microsporus	NEG	elements	inflammation	etiology	True Positive
	HIV, AIDS (CD4 14	Not	amphotericin	Aspergillus		No fungal		Fungal	
52	cells/mcL)	Applicable	В	fumigatus	NEG	elements	Not performed	etiology	True Positive

Supplemental Table S3:

Patient Number	Did Fungal PCR Drive Change in Management in True Positive Cases? (yes/no/other)	Clinical Action/Change	Death (time to)	Cause of death if within 2mos?	Autopsy?
		Medication			
1	Yes	Change/Removal	alive		
		confirmed Medication			
2	No	Choice	=< 1 yr	n/a	No
		confirmed Medication			
3	No	Choice	alive		
4	N/A	no changes	alive		

		Medication			
5	No	Change/Removal	alive		
6	N/A	No Changes			
6	Yes	Medication Change	alive		
6	N/A	Medication Change			
-		Medication			
7	No	Change/Addition	=< 30 days	Fungal infection significant contribution	No
-		confirmed Medication	33 33 73		
8	No	Choice	=< 30 days	Underlying disease	Yes
-		Medication			
9	No	Change/Addition	=< 60 days	Fungal infection significant contribution	Yes
10	N/A	No Change	alive	0	
		Medication			
11	Yes	Change/Addition	alive		
		confirmed Medication			
12	No	Choice	=< 1 yr	n/a	Yes
		Medication			
13	No	Change/Removal	alive		
14	No	Medication Change/	alive		
		Medication	J		
15	N/A	Change/Removal	alive		
		Medication			
16	Yes	Change/Removal	Any Death (>1yr)	n/a	
_		confirmed Medication	, (, ,		
17	No	Choice	alive		
		Medication			
18	Yes	Change/Addition	Any Death (>1yr)	n/a	
		confirmed Medication	, , , ,		
19	No	Choice	alive		
		Medication			
20	No	Change/Removal	Any Death (>1yr)	n/a	
		Medication	, , , ,		
21	No	Change/Removal	alive		
		Medication			
22	No	Change/Addition	alive		
		confirmed Medication			
23	No	Choice	=< 60 days	Fungal infection significant contribution	No
		Confirmed Medication	•		
24	Yes	Choice	Any Death (>1yr)	n/a	

		Medication			No; no physician death note; d/c to home hospice at
25	No	Change/Addition	=< 1 yr	n/a	22 days
		Medication	,		No; no physician death note; transitioned to comfort
26	Yes	Change/Addition	=< 1 yr	n/a	only
	N/A (patient died prior to			Other infection significant contribution	
27	result)	other (see notes)	=< 30 days	Fungal infection significant contribution	Yes
28	N/A	No Change	alive	n/a	
		Confirmed Medication			
29	N/A	Choice	alive	n/a	
30	No	Medication Change/Addition	Any Death (>1yr)	n/a	
31	N/A	Medication Change/Removal	alive	n/a	
32	Unable to determine	confirmed ampho & Vori	Any Death (>1yr)	n/a	
33	N/A	medication Change/Removal	alive	n/a	
34	No	Medication Change/Addition	=< 1 yr	n/a	No
35	N/A		alive	n/a	
36	N/A	medication Change/Removal	=< 1 yr	Not documented/Unknown	No
37	No	Medication Change/Addition	alive	n/a	
38	Yes	confirmed Medication Choice	alive	n/a	
20	Yes* (supported interpretation of culture	No outifunction			
39	as bystander)	No antifungals given	alive	n/a	
40	Yes	Medication Change/addition	alive	n/a	No

41	N/A	no Change	=< 1 yr	Not documented/Unknown	No
42	N/A	no change	=< 1 yr	n/a	Yes
		medication			
43	No	Change/Removal	Any Death (>1yr)	n/a	
		medication			
44	No	Change/Addition	=< 30 days	Fungal infection significant contribution	No
		Medication			
45	No	Change/addition	=< 30 days	Fungal infection significant contribution	Yes
46	N/A	n/a	Any Death (>1yr)	n/a	
	First identified by				
	Aspergillus fumigatus-				
	specific PCR, ordered	Medication			
47	concurrently	Change/Addition	alive		
	First identified by				
	Aspergillus fumigatus-				
	specific PCR, ordered	medication change			
48	concurrently	(posa to vori)	=< 1 yr	n/a	No
49	N/A	No Change	alive		
50	No	No Change	=< 30 days	Other infection significant contribution	No
	First identified by				
	mucormycete-specific				
	PCR, ordered	Confirmed Medication			
51	concurrently	Choice	=< 30 days	Fungal infection significant contribution	No
	First identified by				
	Aspergillus fumigatus-				
	specific PCR, ordered	Medication			
52	concurrently	Change/Addition	=< 30 days	Fungal infection significant contribution	No