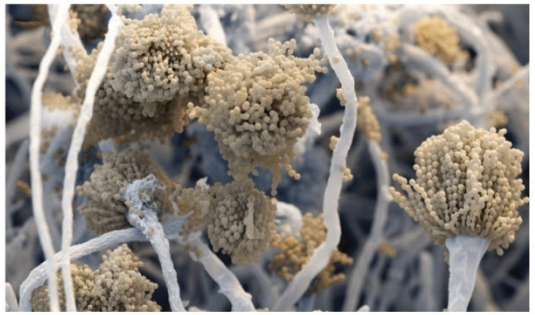
Monitorization, Separation and Quantification of Antifungals used for Invasive Aspergillosis Treatment by High Performance Thin Layer Chromatography

M. P. Domingo, M. Vidal, J. Pardo, A. Rezusta, L. Roc, M.J. Revillo, E.M. Galvez

Invasive Aspergillosis



Research Highlights (2007)
Nature 445, 798 - 799

Opportunistic hospital infection

Causative Agent: *Aspergillus fumigatus* (90%)

Risk factor: Immunosuppression (neutropenia)

* Chemotherapy in cancer (leukemia)
* Transplanted

Mortality: 20-80%

due to the lack of an early diagnosis

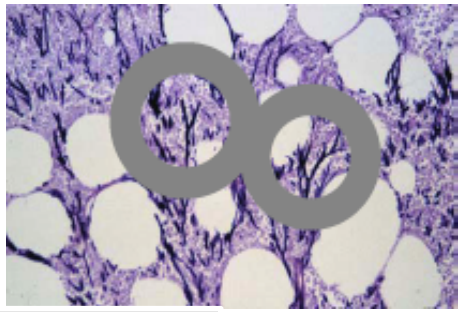
Invasive Aspergillosis: Criteria for Diagnosis

All Patients : neutropenia, fever, etc
several infection evidences

Proven: Hyphae in Biopsy or BAL
A.f growth in vitro
Röntgen/CT Evidence

Probable: Hyphae in Biopsy or BAL
Röntgen/CT Evidence
A.f. Antigen (galactomannan) ELISA

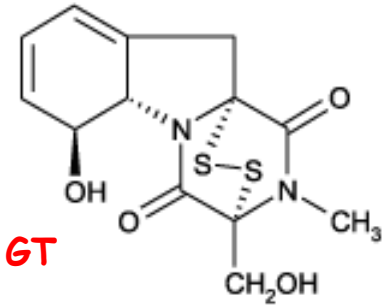
Possible: Röntgen/CT Evidence



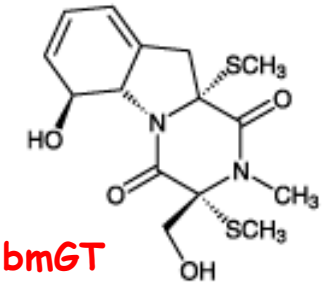
Late non specific diagnosis → High mortality (20-80%)

Invasive Aspergillosis: Criteria for Diagnosis

BIOMARKERS



GT



bmGT

Fungal metabolities → Early Diagnosis

HPTLC system → Toxins in sera

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journal homepage: www.elsevier.com/locate/diagmicrobio

Bis(methyl)gliotoxin proves to be a more stable and reliable marker for invasive aspergillosis than gliotoxin and suitable for use in diagnosis☆

Maria P. Domingo[a], Cristina Colmenarejo[b,1], Luis Martínez-Lostao[c,1], Arno Müllbacher[d], Carmen Jarne[a], María J. Revillo[b], Pilar Delgado[e], Lourdes Roc[b], Jacques F. Meis[f,g], Antonio Rezusta[b], Julian Pardo[c,h,*,2], Eva M. Gálvez[a,2]

Invasive Aspergillosis: Criteria for Diagnosis

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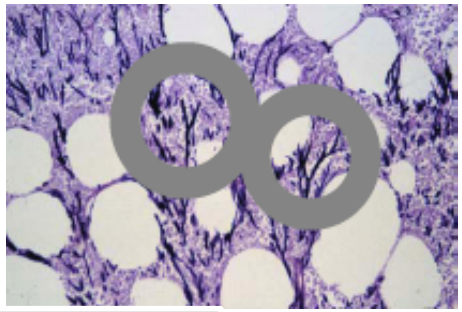


Late non specific diagnosis → High mortality (20-80%)
High cost (prophylaxis)

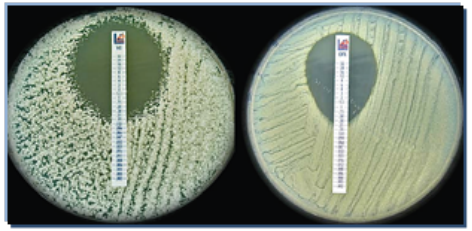
Monitoring drug system to check the antifungal treatment taken by patients.

ANTIFUNGALS

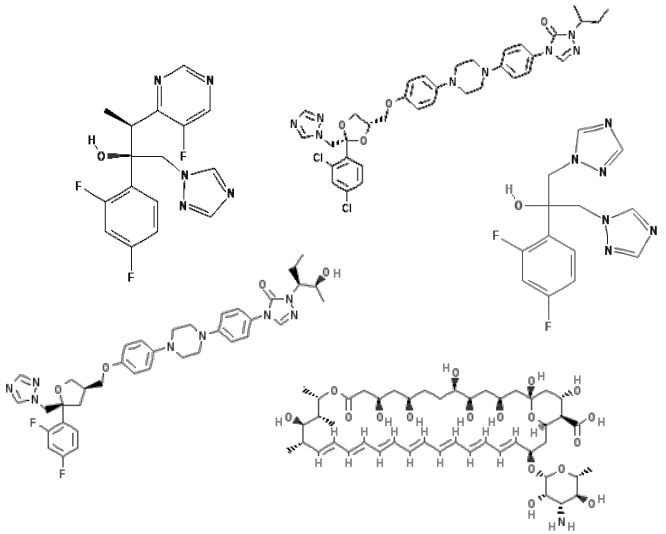
Antifungals are often used in patients at risk of Invasive Aspergillosis such as prophylaxis and treatment.

There is no optimal therapy for Invasive Aspergillosis.

Most commonly prescribed antifungals are:



Voriconazole
Itraconazole
Fluconazole
Posaconazole
Anfotericin B

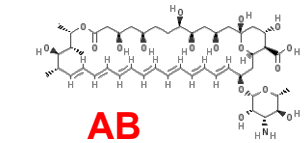


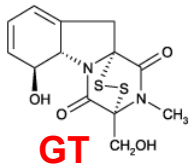
Sometimes a combination of antifungals is used to improve outcomes.

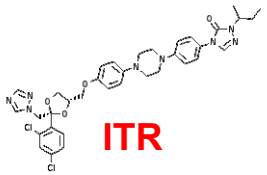
Therapeutic drug monitoring can help to minimize the risk of toxicity and maximize the efficacy.

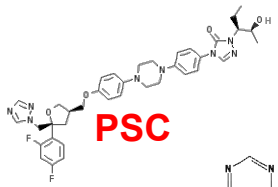
ANTIFUNGALS and BIOMARKERS: Detection by HPTLC-UV

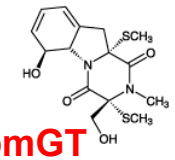
methodology

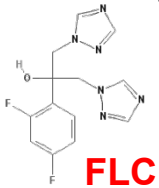


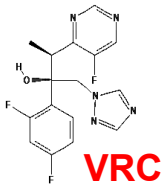




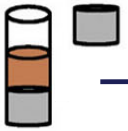




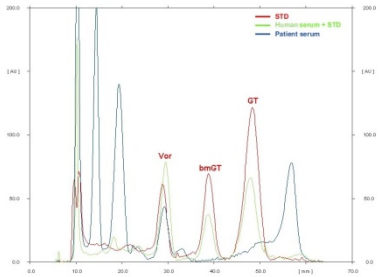




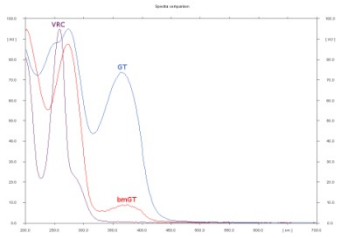
DCM



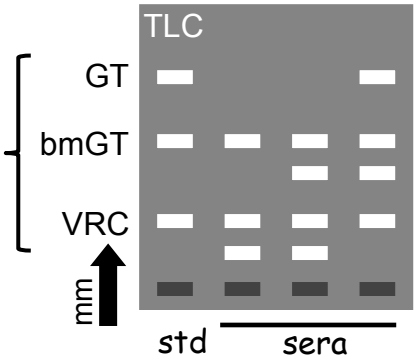
extraction



UV-scanning



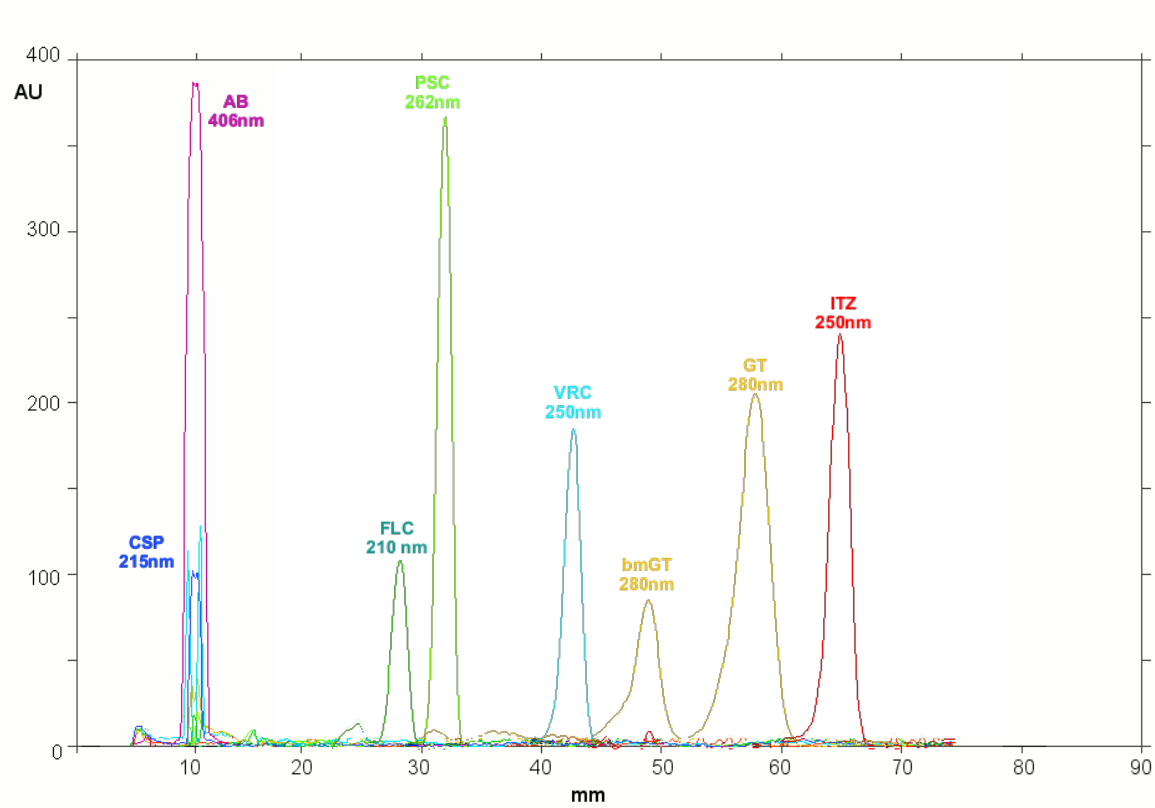
Spectrum



Separation

MONITORING SYSTEM

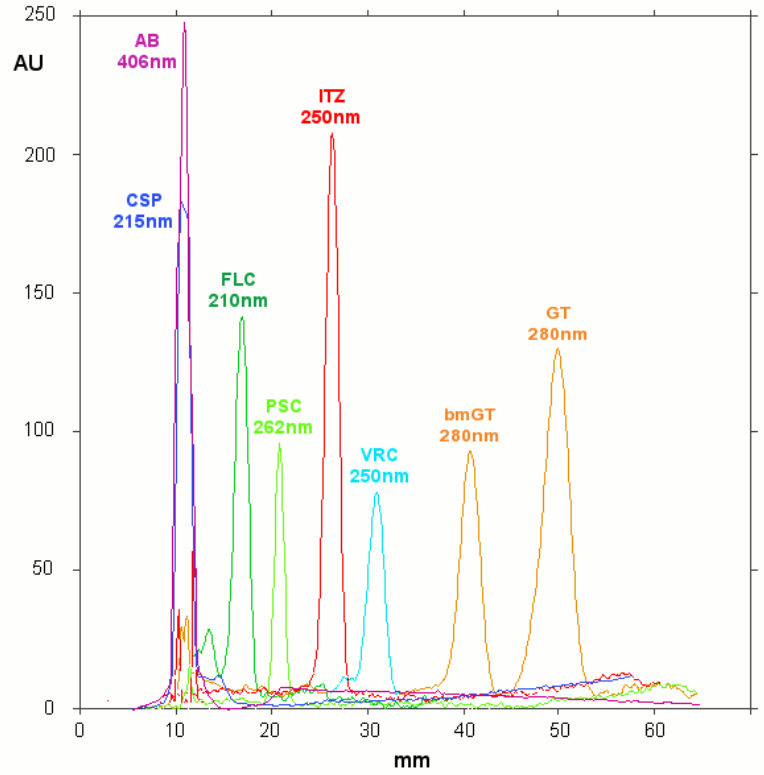
We have obtained two different separation methods for some antifungals.

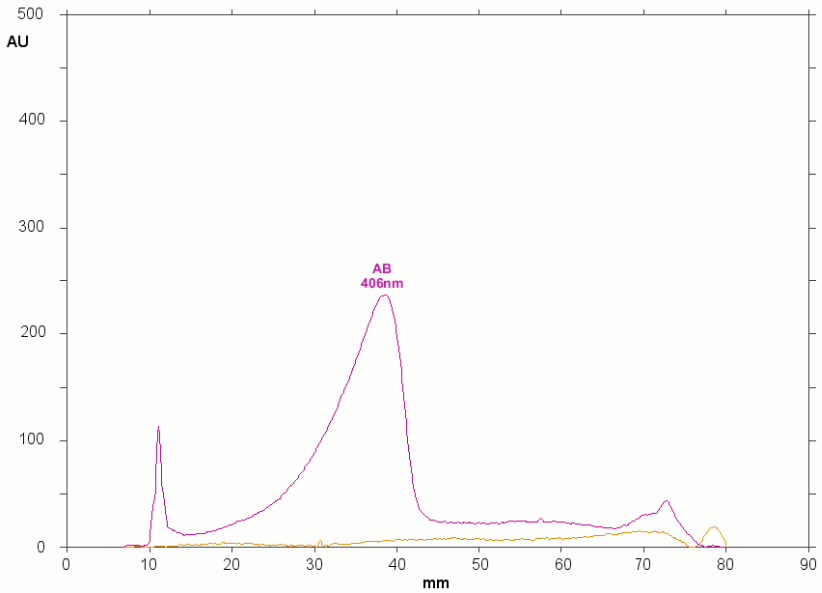


Acetone:Toluene:MeOH

Different antifungals are detected in the same analysis only changing the wavelength.

Separation





Use sequential elution with:

1. THF:C7:ACN
2. MeOH

to obtain the AB separation.

MONITORING SYSTEM

These two kind of separation sequence could be use to have screening analysis and the treatment taken by patients.

Recovery

SEPARATION SYSTEM

Similar recovery percentage than in toxins analysis was obtained to Voriconazole, Itraconazole and Fluconazole.

Best extraction system with DCM as extracted solvent.

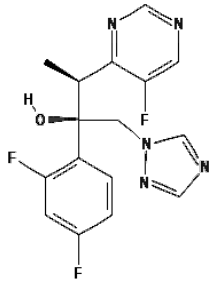
		GT			bmGT		
	Nivel	mean	*sd*	*RSD*	mean	*sd*	*RSD*
DCM							
	0,05ug	77.97	*14.63*	***18.77***	75.95	*11.71*	***15.42***
	0,5ug	94.34	*7.20*	***7.63***	96.10	*8.19*	***8.53***
	5 ug	91.79	*9.10*	***9.91***	94.65	*6.09*	***6.43***
AcN							
	0,05ug	77.04	*36.00*	***46.73***	82.03	*18.72*	***22.82***
	0,5ug	51.00	*9.72*	***19.07***	60.08	*5.04*	***8.38***
	5 ug	35.87	*2.78*	***7.75***	50.09	*8.69*	***17.36***

		Fluconazole			Posaconazole		
	Nivel	mean	*sd*	*RSD*	Nivel	mean	*sd*	*RSD*
DCM								
	0,1ug	65.35	*12.35*	***18.90***	0,02ug	-	-	-
	0,4ug	70.17	*11.41*	***16.26***	0,15ug	-	-	-
	0,8ug	89.86	*7.34*	***8.17***	0,3ug	-	-	-
AcN								
	0,1ug	-	-	-	0,02ug	-	-	-
	0,4ug	-	-	-	0,15ug	34.52	*14.68*	***42.54***
	0,8ug	66.77	*6.42*	***9.61***	0,3ug	53.46	*6.42*	***12.00***

		Voriconazole			Itraconazole		
		mean	*sd*	*RSD*	mean	*sd*	*RSD*
DCM							
	0,05ug	81.07	*2.01*	***2.48***	44.01	*1.44*	***3.27***
	0,5ug	95.56	*3.07*	***3.21***	46.30	*2.64*	***5.69***
	5 ug	88.57	*1.11*	***1.25***	22.14	*5.38*	***24.28***
AcN							
	0,05ug	33.03	*14.61*	***44.23***	80.40	*7.78*	***9.68***
	0,5ug	25.25	*5.07*	***20.06***	85.55	*12.34*	***14.43***
	5 ug	20.01	*5.03*	***25.14***	85.28	*5.28*	***6.19***

		Caspofungine			Anfotericin B		
	Nivel	mean	*sd*	*RSD*	Nivel	mean	*sd*	*RSD*
DCM								
	0,005ug	-	-	-	0,02ug	-	-	-
	0,075ug	-	-	-	0,15ug	-	-	-
	0,175ug	-	-	-	0,3ug	-	-	-
AcN								
	0,005ug	-	-	-	0,02ug	-	-	-
	0,075ug	-	-	-	0,15ug	-	-	-
	0,175ug	-	-	-	0,3ug	1.91	*1.09*	***56.94***

Voriconazole



Triazole antifungals are used for the prophylaxis as well as for the treatment in Invasive fungal infection.
Voriconazole is first chose for treating Invasive Aspergillosis.


A. terreus

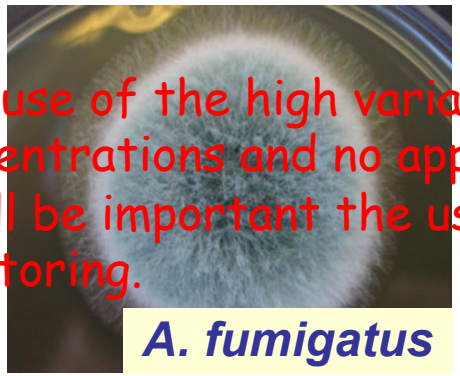
Voriconazole is an extended-spectrum triazole antifungal with activity against a wide variety of pathogens, including aspergillus and Candida.

Optimum Voriconazole trough levels are between 1 and 5 mg/L but it is unclear whether these levels are reached with currently dosing schedules.


A. nidulans

Adverse effects as neurological toxicity are related with voriconazole dosage >5 mg/L.

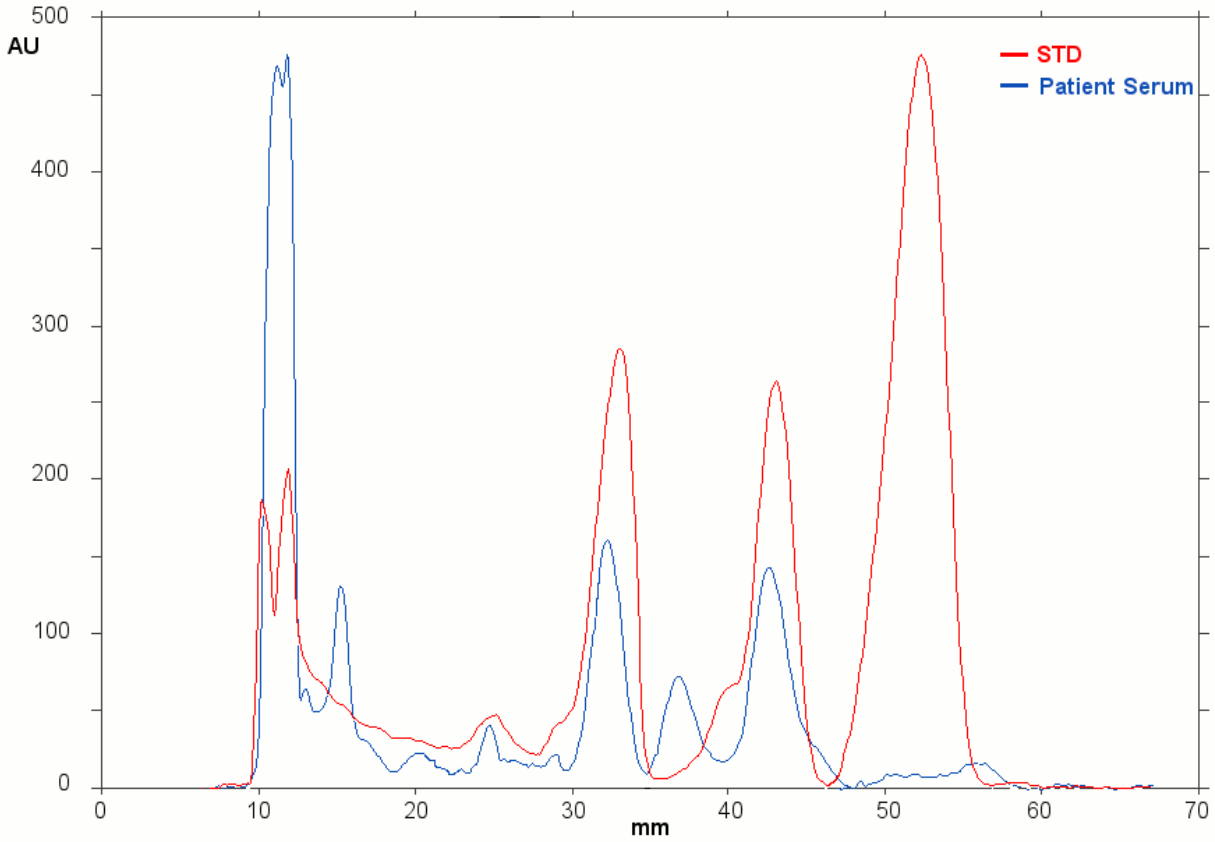

A. flavus


A. fumigatus

Because of the high variability in through concentrations and no apparent relationship with dose, it will be important the use of therapeutic drug monitoring.


A. niger

Detection



No interferences by endogenous components was noted.

This system allowed analyze at same time antifungals and disease biomarkers.

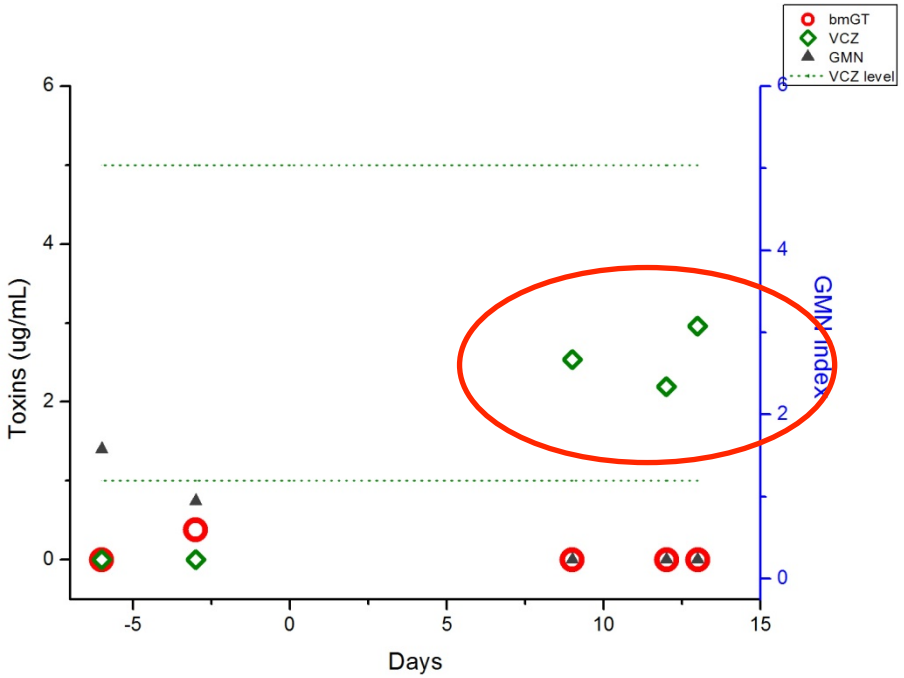
Monitoring infection development and treatment at same time.

Treatment Drug Monitoring

Patients treated with VRC.

CASE 1

In these figures, it is showed the VRC and bmGT quantities, GMN index (other disease marker) and optimal VCZ levels.



Loading dose of 400mg orally twice a day

Maintenance dose of 200mg twice a day.

No prophylaxis treatment, started to treat because of the symptoms.

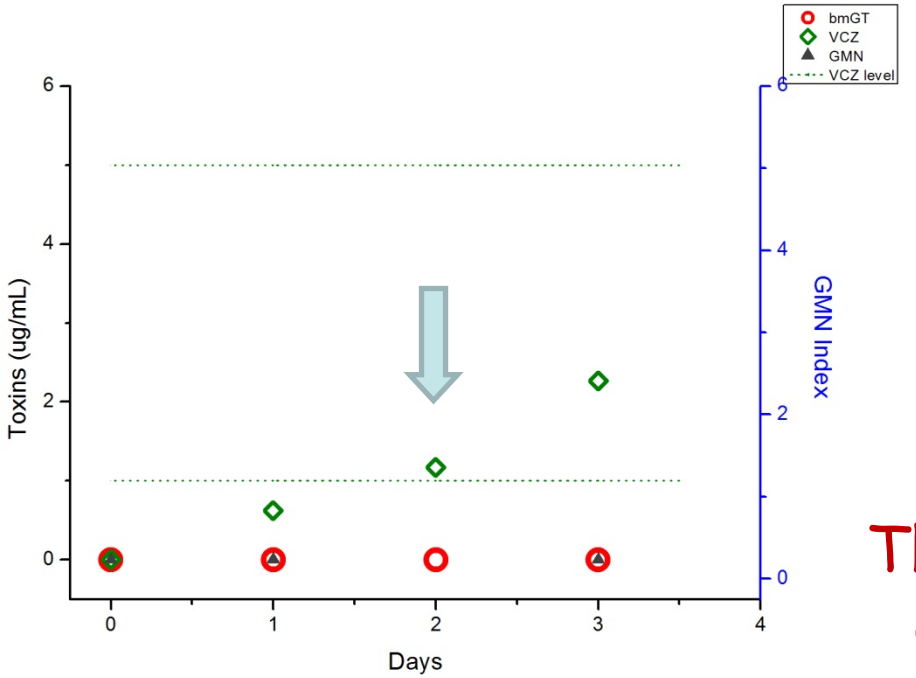
Voriconazole concentration monitoring was made on day 9, 12 and 13.

Maintained in therapeutic level.

Treatment Drug Monitoring

CASE 2

Voriconazole monitorization from day 0 to day 3.



Loading dose of 400mg orally twice a day

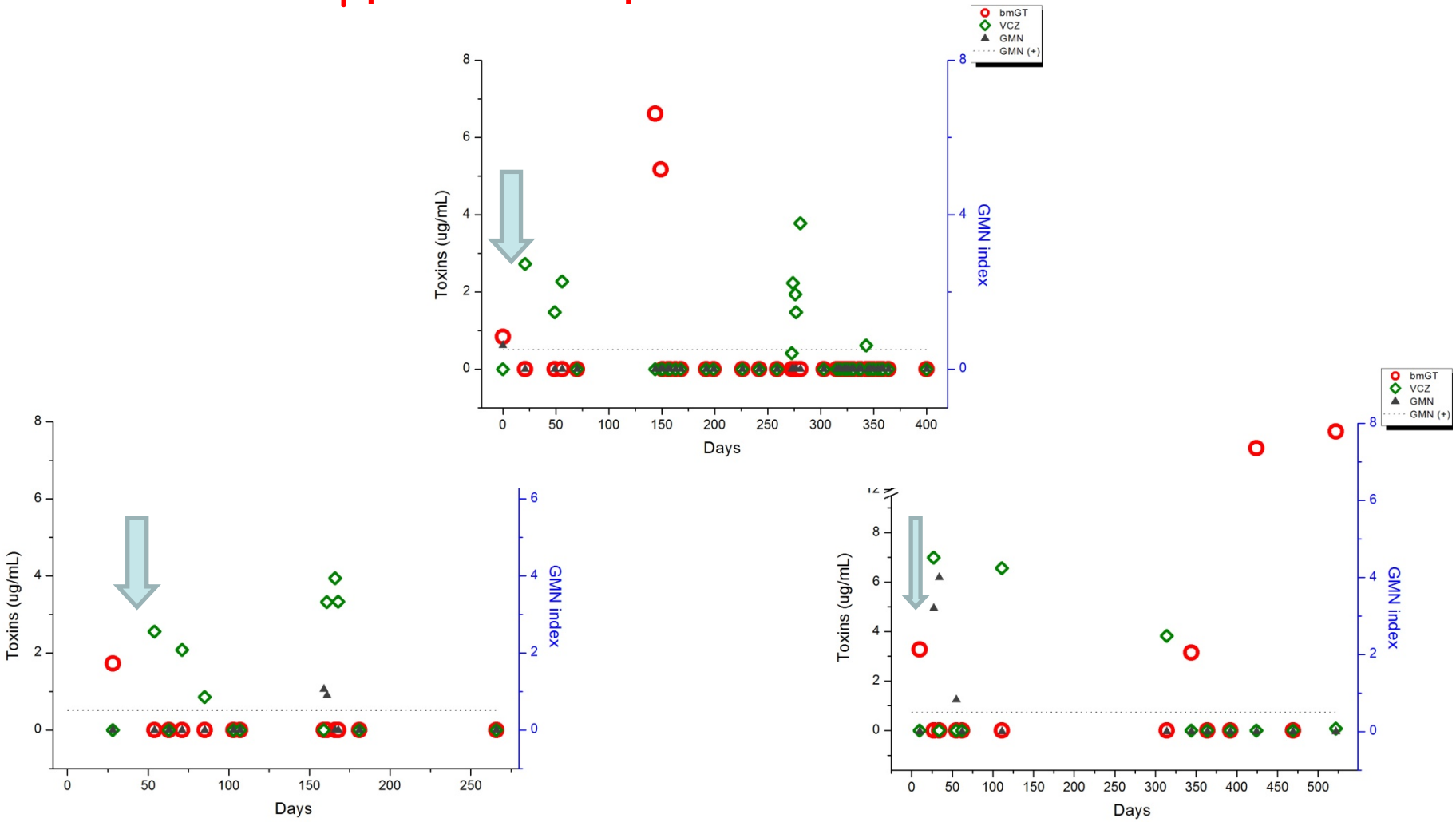
Maintenance dose of 200mg twice a day.

Therapeutic dose is reached at second treatment day.

Treatment Drug Monitoring

Other voriconazole treated patients are checked.

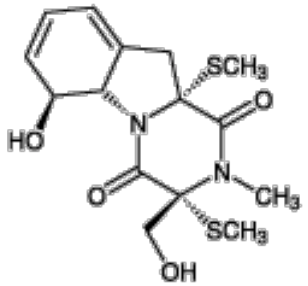
bmGT disappear while patient is treated with VRC.



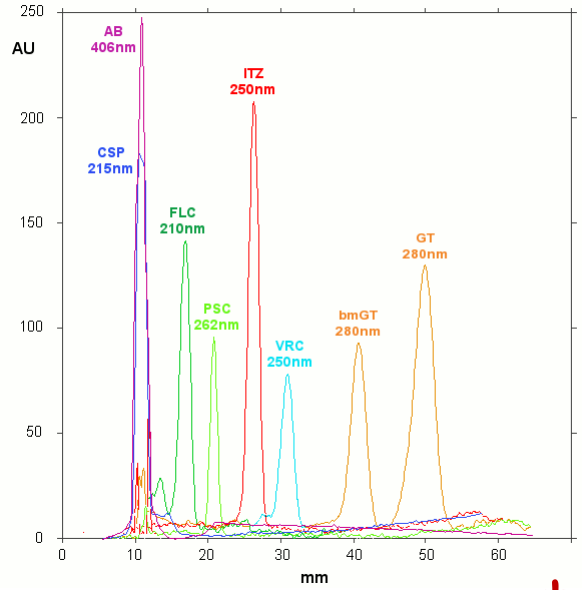
SUMMARY

We have established:

A system that allow us to monitor:



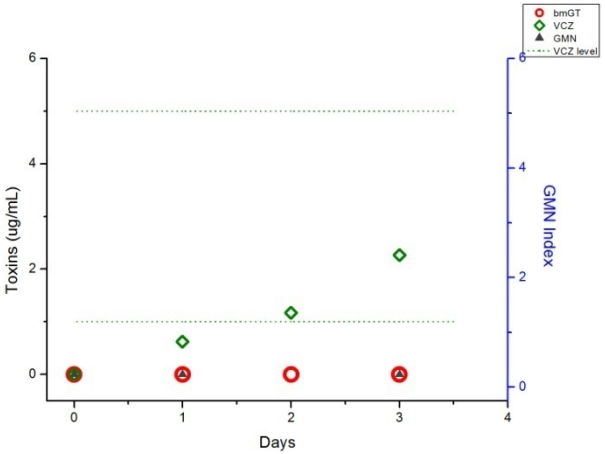
the infection by BIOMARKER,



treatment by the SCREENING

treatment efficacy by

DRUG MONITORING.



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