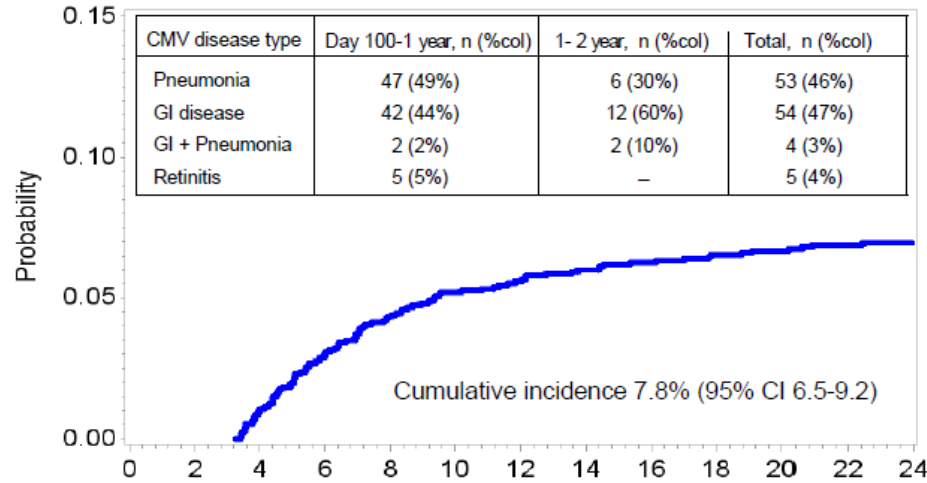


CMV Dried Blood Spot Testing

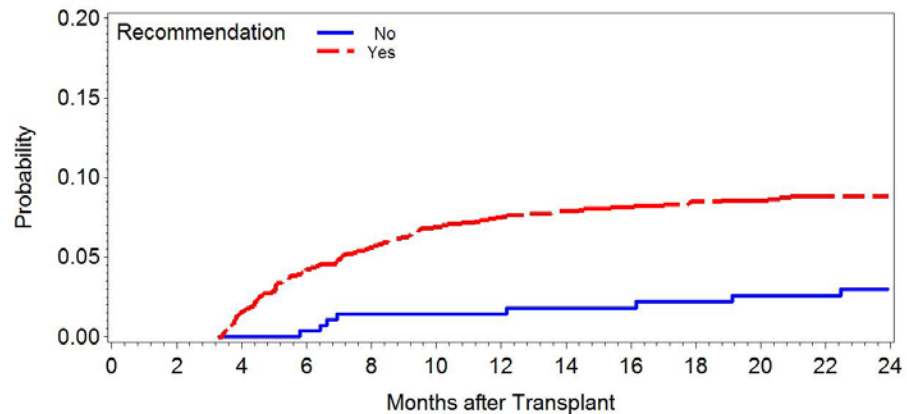
Michael Boeckh, MD
Member, Vaccine and Infectious Disease Division
Head, Infectious Disease Sciences Program
Fred Hutchinson Cancer Research Center
Professor, Department of Medicine, University of Washington
Seattle, Washington, USA

Late CMV Disease

Continuing Challenge – Who is at Risk?



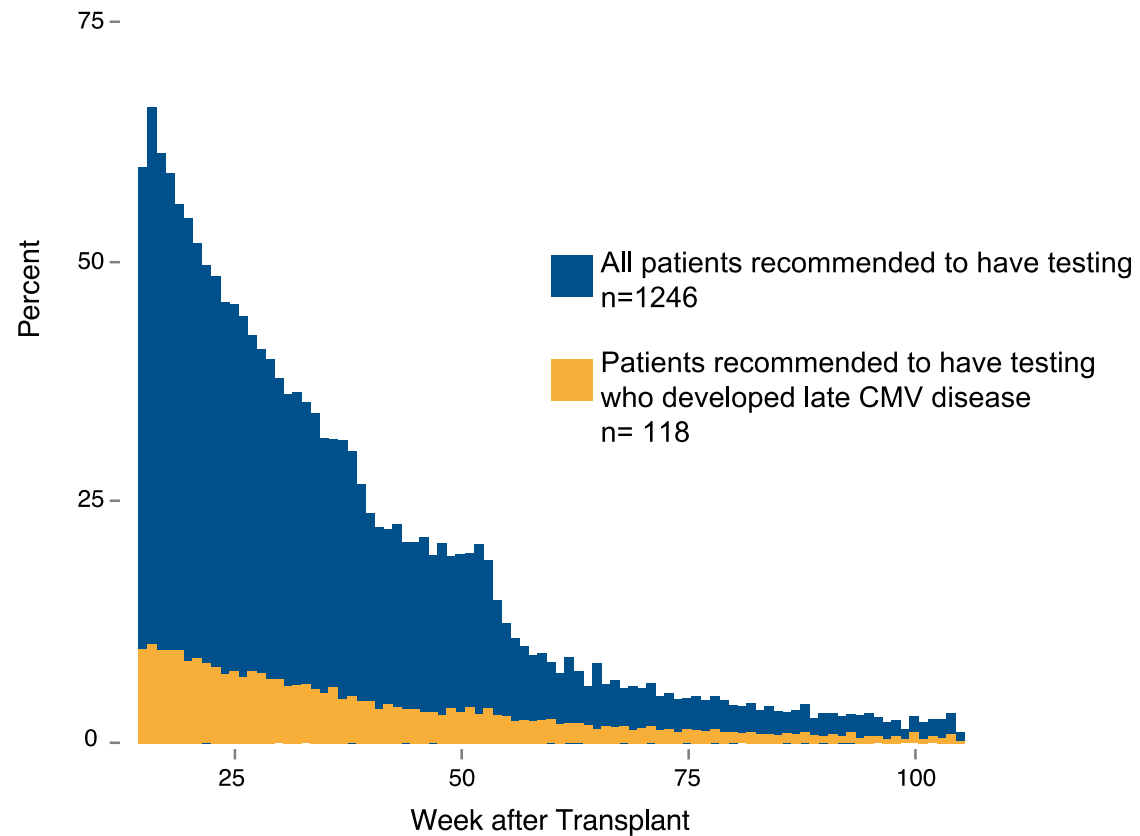
Cumulative Incidence of 1st Late CMV Disease
Among 100 Days Survivors only (N=1526)

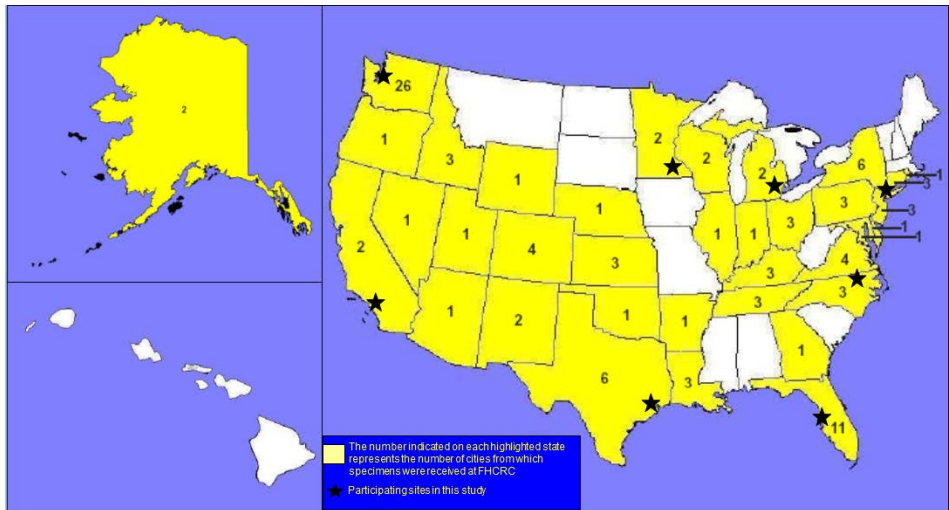


Covariates		Multivariable	
		Adjusted HR (95% CI)	P value
CMV serostatus	D-/R+	1	
	D+/R+	1.29 (0.87–1.89)	0.202
	D+/R-	0.59 (0.28–1.26)	0.175
Acute GVHD grade 3-4		1.14 (0.70–1.85)	0.595
CMV viremia before day 100		2.15 (1.31–3.52)	0.002
CMV disease before day 100		1.92 (1.05-3.54)	0.035
Lymphocyte count at day 100	≥ 300 cells/mm ³	1	
	< 300 cells/mm ³	2.11 (1.28-3.49)	0.003
	Missing	0.90 (0.13-6.51)	0.920
Corticosteroid treatment after day 100		7.12 (3.76–13.50)	<0.001
CMV Viremia after day 100		3.85 (2.57–5.76)	<0.001

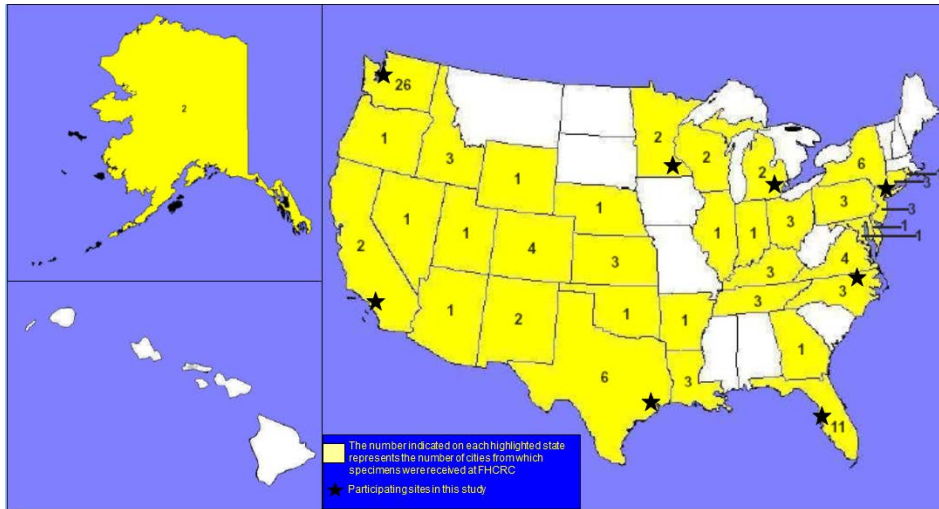
Surveillance Adherence

Proportion of patients with CMV surveillance test by week after day 100

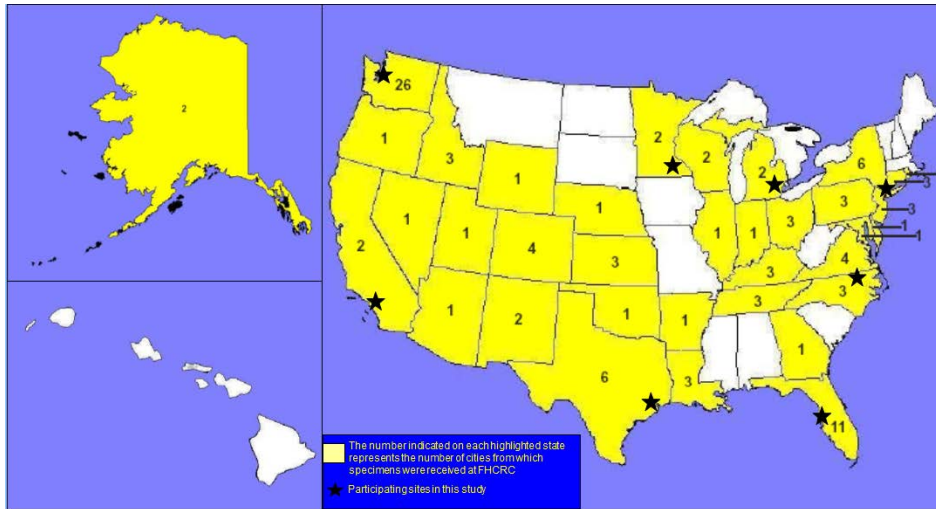




Late CMV Disease: Challenges



Late CMV Disease: Challenges



Patient-based Testing

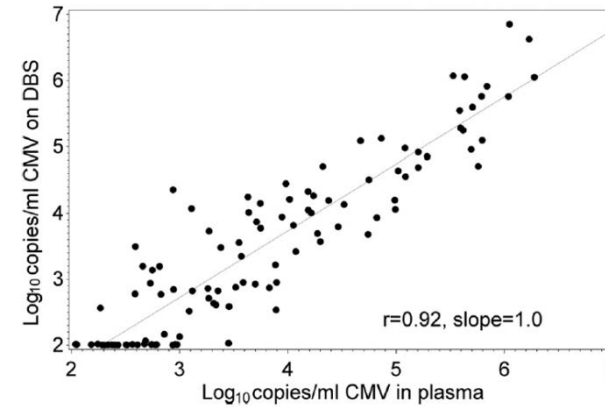


FIG 1 Comparison of CMV viral load in dried blood spot and plasma samples.

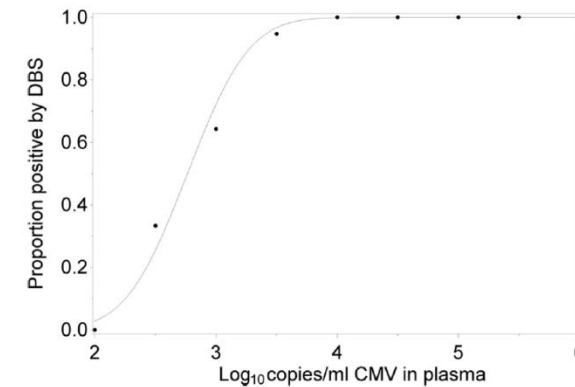


FIG 3 Probit modeling of the likelihood of DBS positivity as a function of plasma CMV load.

Assay Evolution

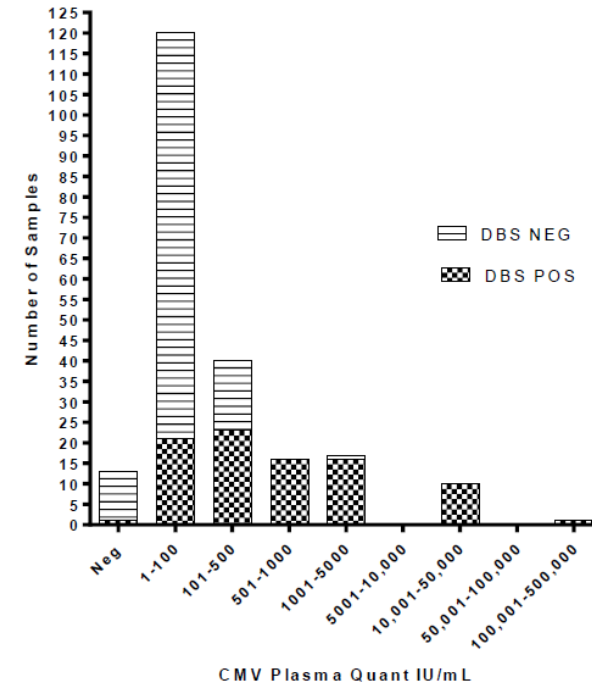
DESCRIPTION	Initial Procedure (Limaye et al. 2013)	CURRENT PROCEDURE CMV On The Go
Filter paper used	Whatman FTA Elute cards	Whatman 903 Protein Saver cards
Size of each punch	3 mm in diameter	6 mm in diameter
Number of punches used for DNA extraction	4	2
DNA extraction procedure	washed the DBS twice with 1 ml of water, then added 100 ul of 5% Chelex and incubated the vials at 95°C for 30 minutes to elute the DNA from the paper	pre-treat DBS with proteinase K, spike internal control template, perform DNA extraction using Promega Maxwell 16 Viral Total Nucleic Acid Purification Kit on Promega Maxwell 16 MDx Instrument.
PCR master mix	QuantiTect Multiplex PCR mix with ROX	QuantiTect Multiplex PCR NoROX mix
PCR Machine	7900HT sequence detection system	QuantStudio 7 flex real-time PCR system
IU conversion	1 IU= 4 copies (AcroMetrix)*	1 IU=1.4 copies (WHO)
Internal control template	Spiked into PCR master mix	Spike into extraction lysis buffer
Internal control primers and probe	EXO	EXO BS

CMV on the Go

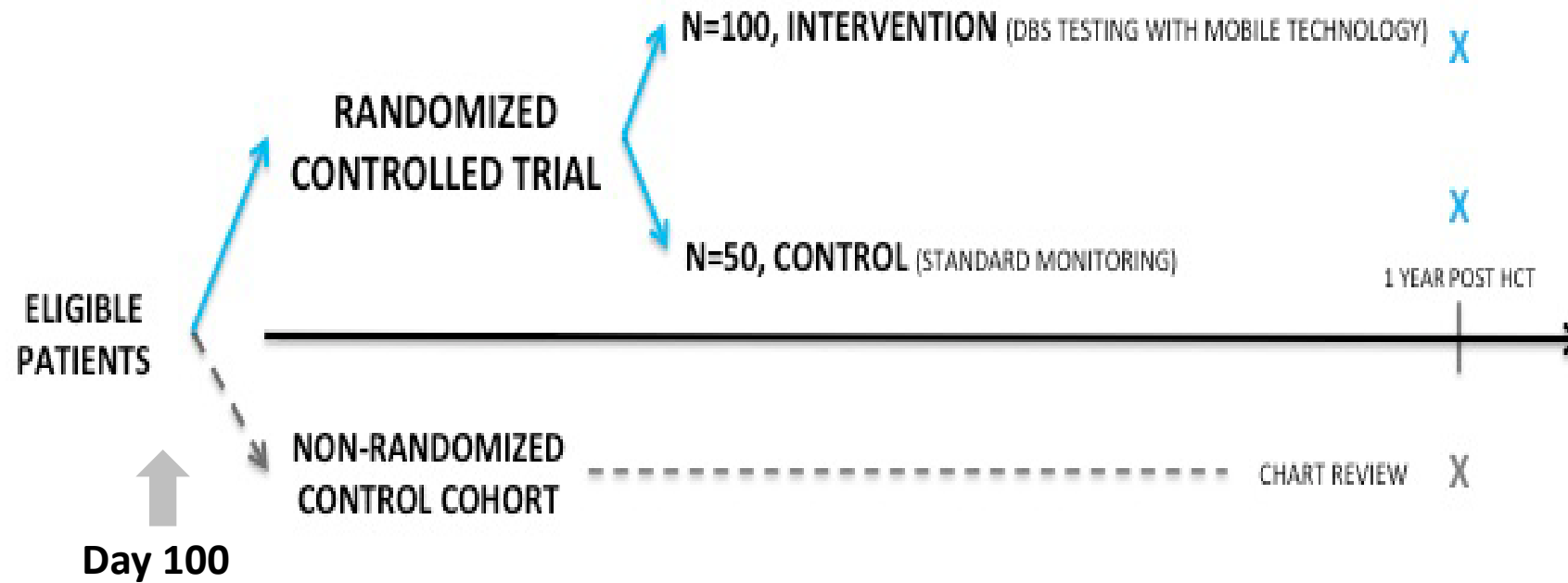
Left-over blood from clinical testing (UW/SCCA)

N=217	Plasma Pos	Plasma Neg
DBS Pos	87	1
DBS Neg	117	12

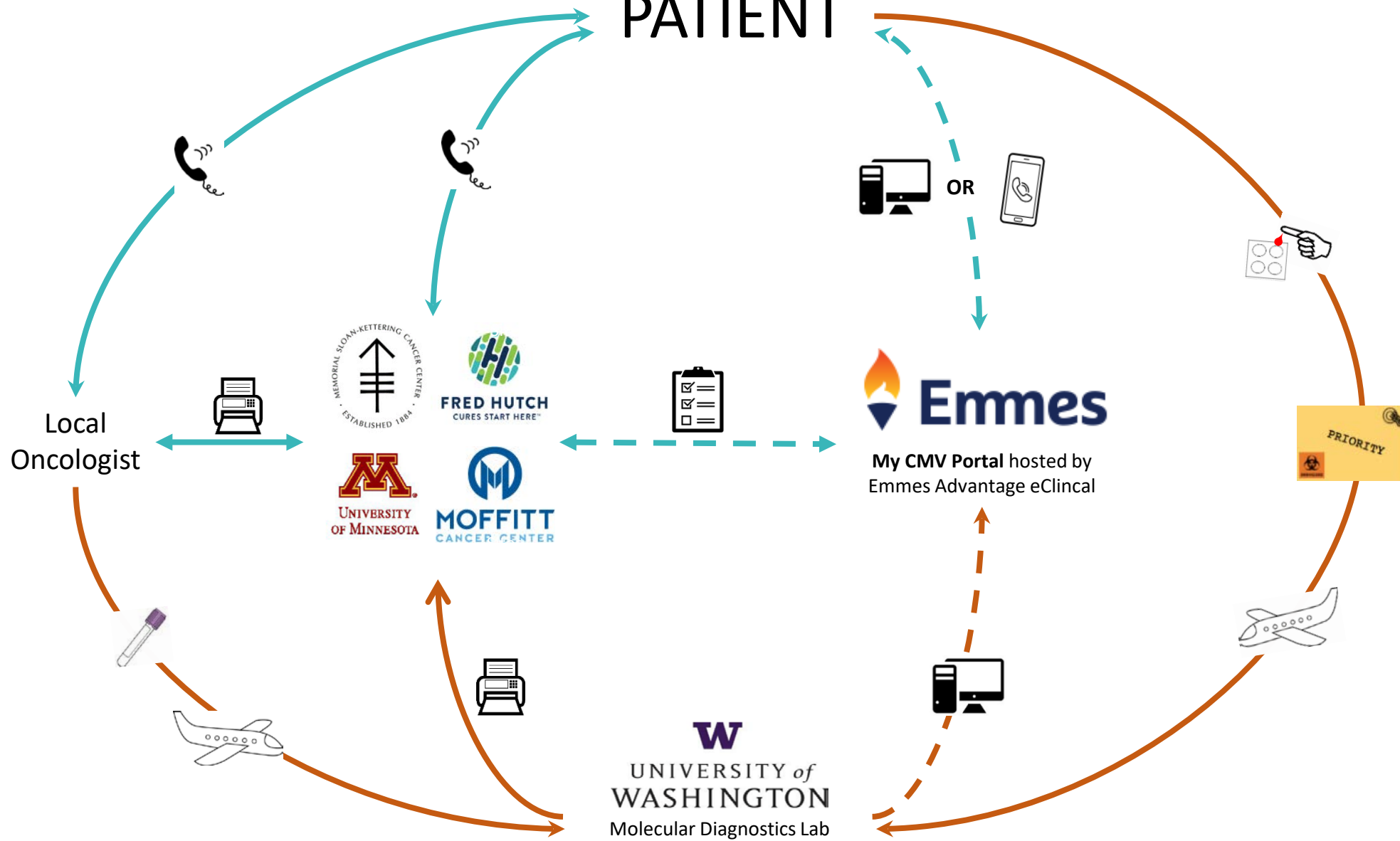
N=204	Plasma Pos	Plasma Neg
WB Pos	116	1
WB Neg	76	11



Proposed trial for DBS CMV testing after day 100



PATIENT



— Bi-directional Communication

— Uni-directional Communication

- - - Bi-directional Communication with Password Protected Secure Access

- - - Uni-directional Communication with Password Protected Secure Access

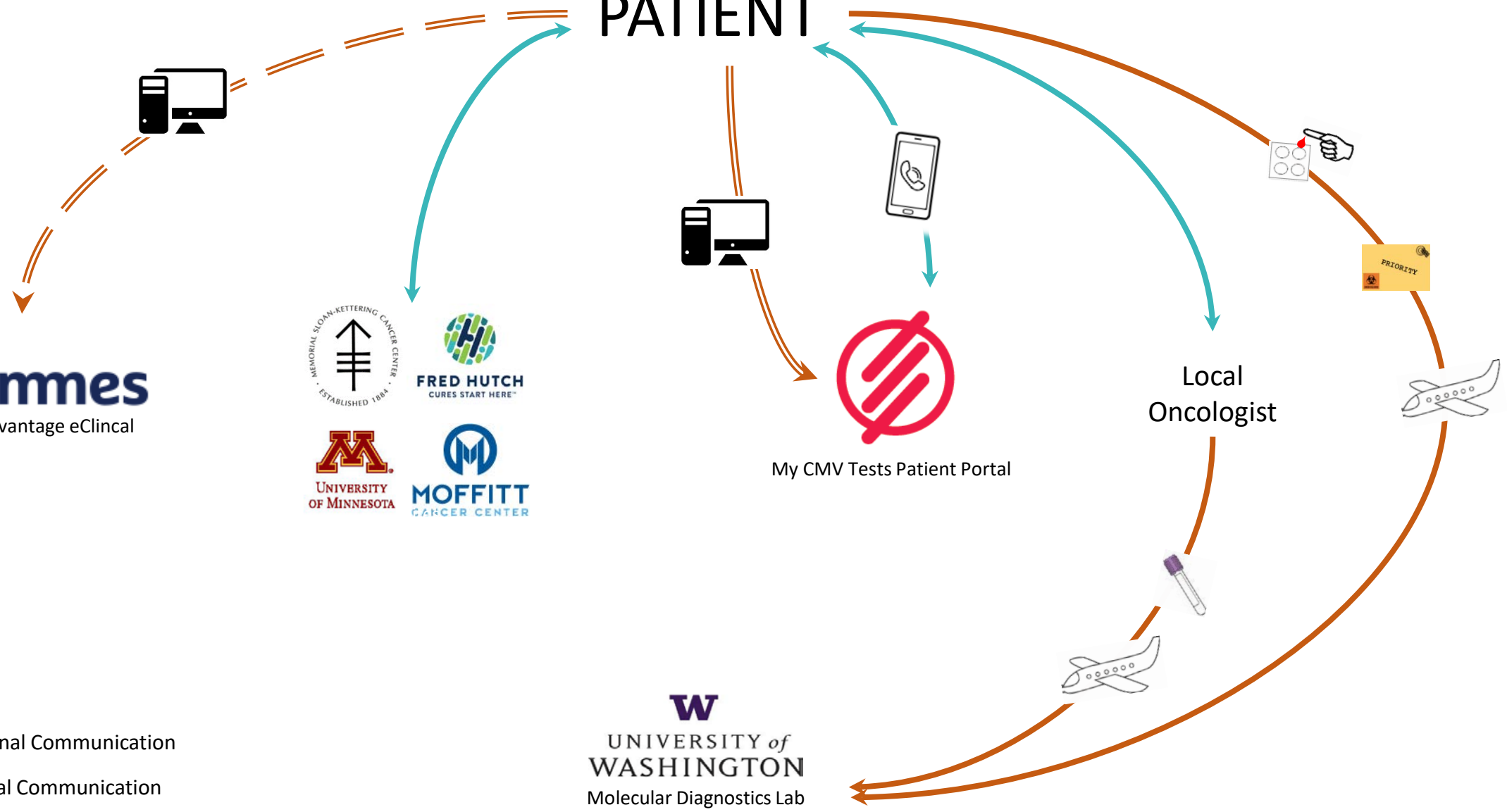
PATIENT



Local Oncologist



- Uni-directional Communication
- Bi-directional Communication
- Password Protected Secure Access
- TBD







PATIENT

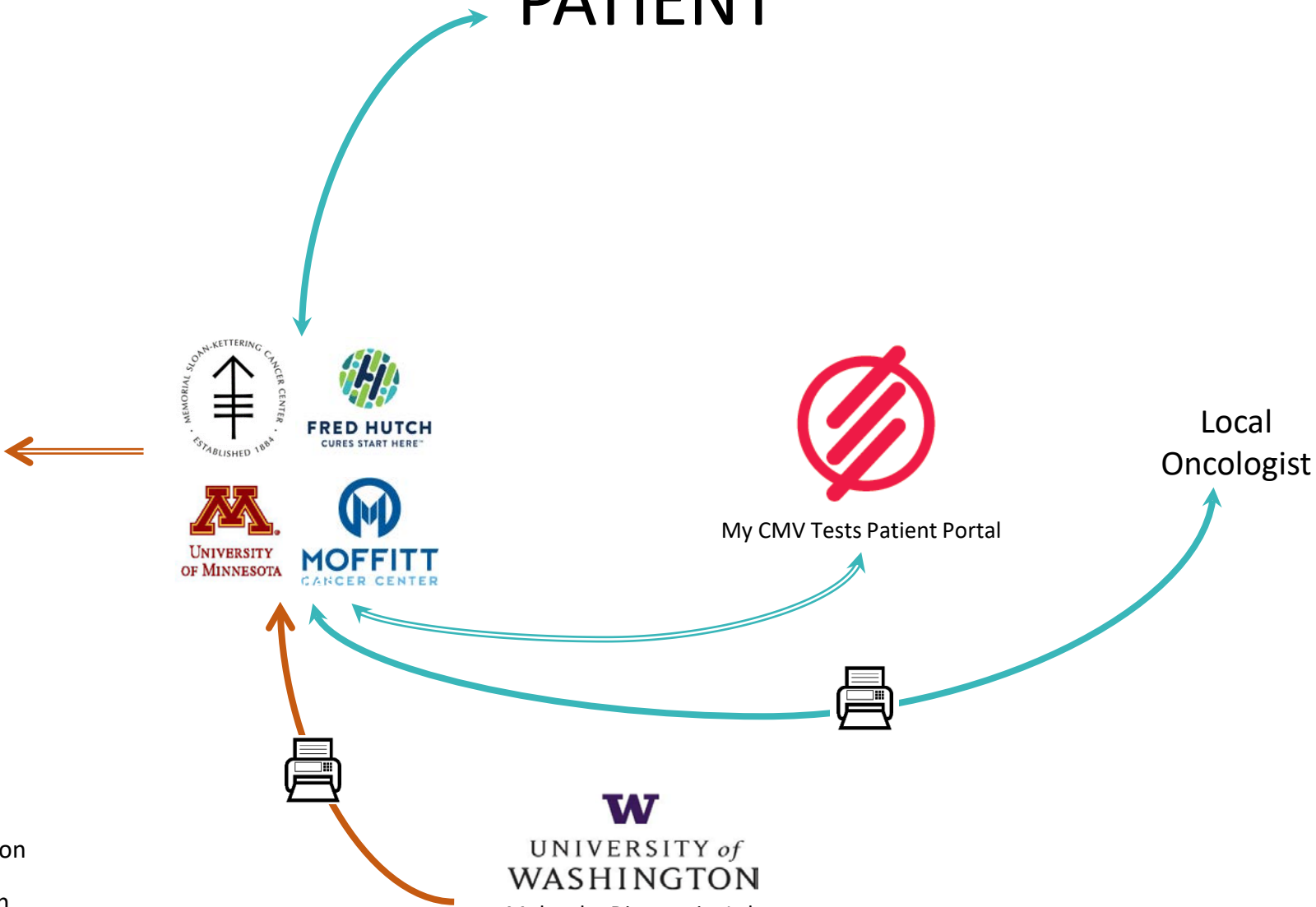


My CMV Tests Patient Portal

Local
Oncologist



-  Uni-directional Communication
-  Bi-directional Communication
-  Password Protected Secure Access
-  TBD

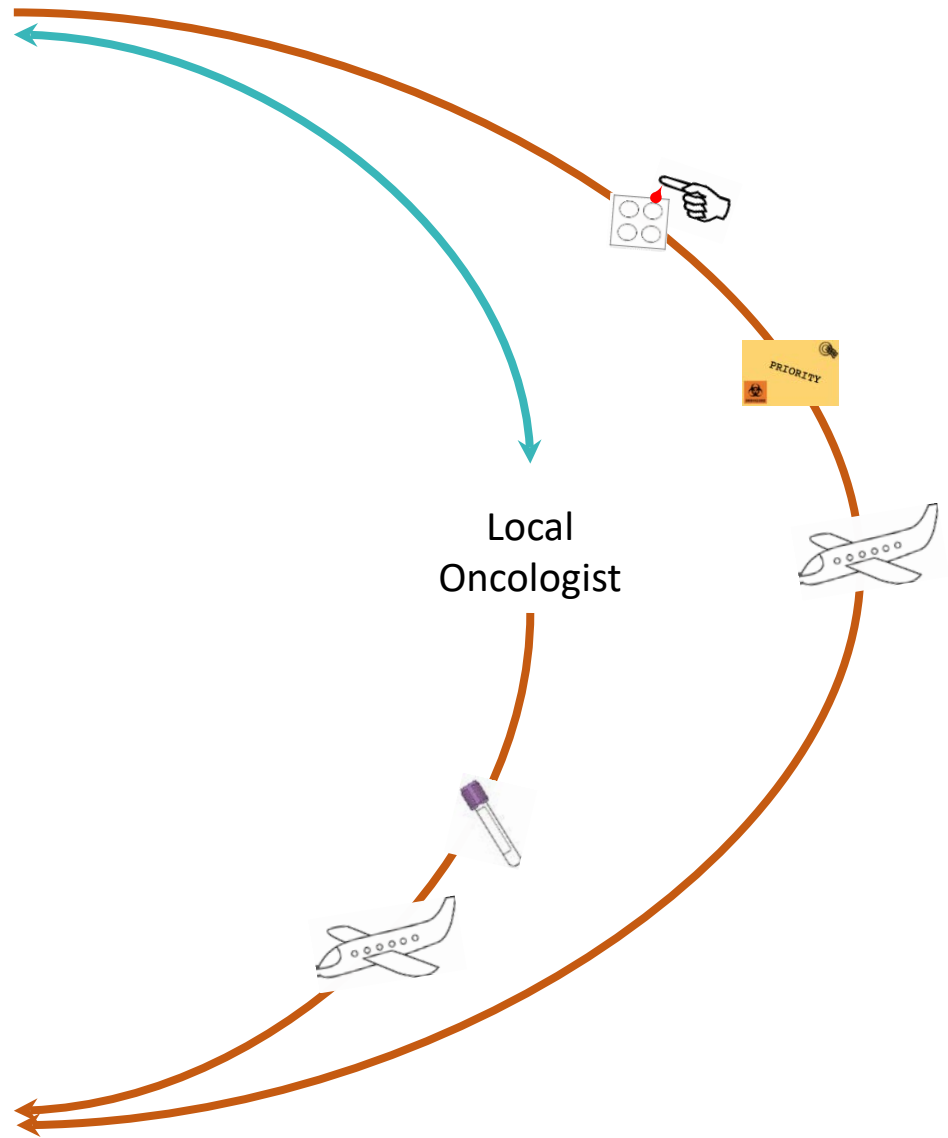


PATIENT



Local Oncologist





- Uni-directional Communication
- Bi-directional Communication
- Password Protected Secure Access
- TBD

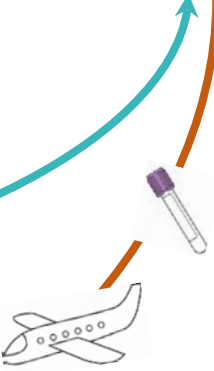


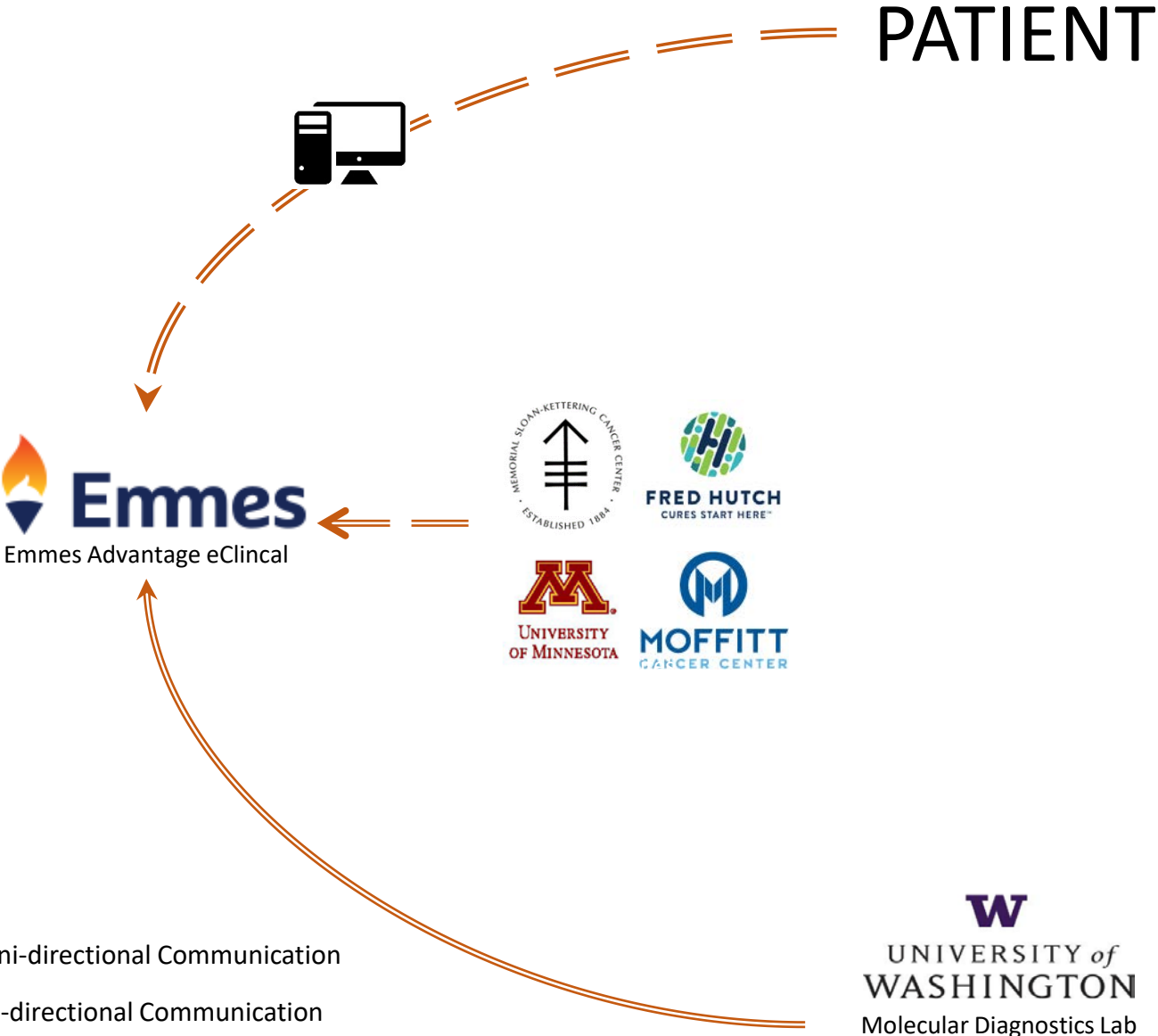
PATIENT





Local Oncologist



-  Uni-directional Communication
-  Bi-directional Communication
-  Password Protected Secure Access
-  TBD

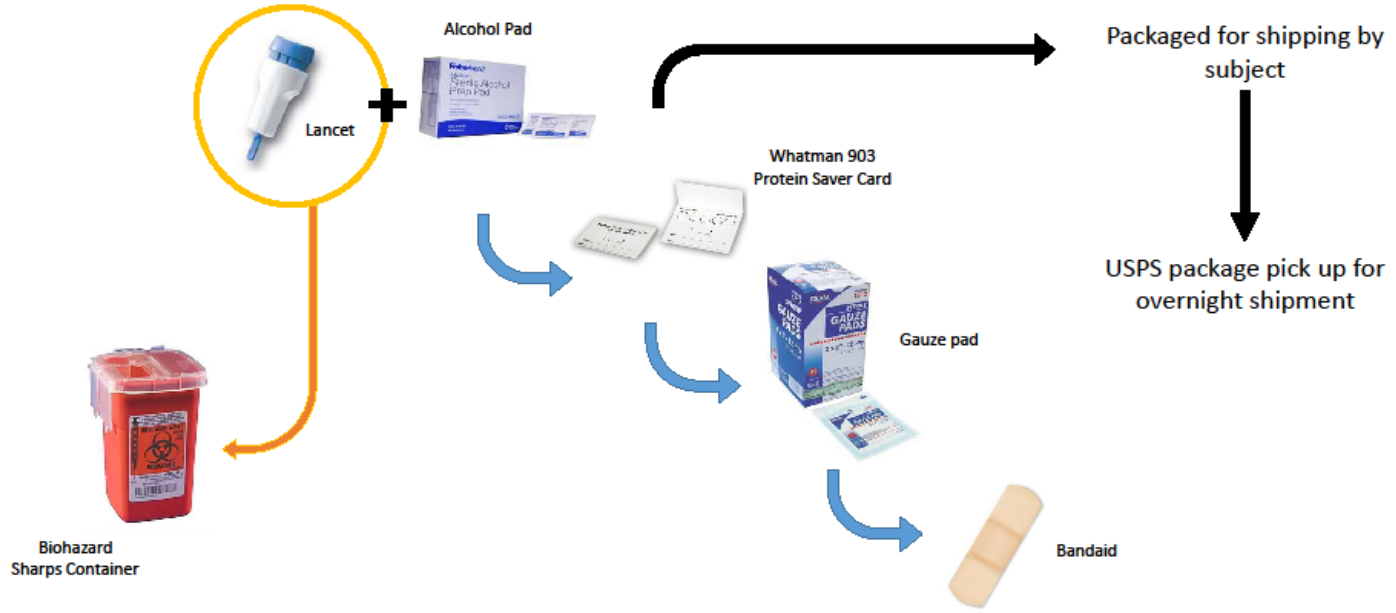




-  Uni-directional Communication
-  Bi-directional Communication
-  Password Protected Secure Access
-  TBD

Testing Procedure

Test CMV On the Go—DBS Component



Acknowledgments

Ajit Limaye

Margaret Green

Louise Kimball

Cindy Hirano

James Reith

Lisa Chung

Hu Xie

Wendy Leisenring

Amalia Margaret

Zach Stednick

MeeiLi Huang

Linda Cook

Keith Jerome

Schematic of Study Design

