Effect of μ-opioid receptor (MOR) engagement on residual immune activation in HIV-infected individuals with OUD receiving suppressive antiretroviral treatment (ART).

Livio Azzoni, The Wistar Institute

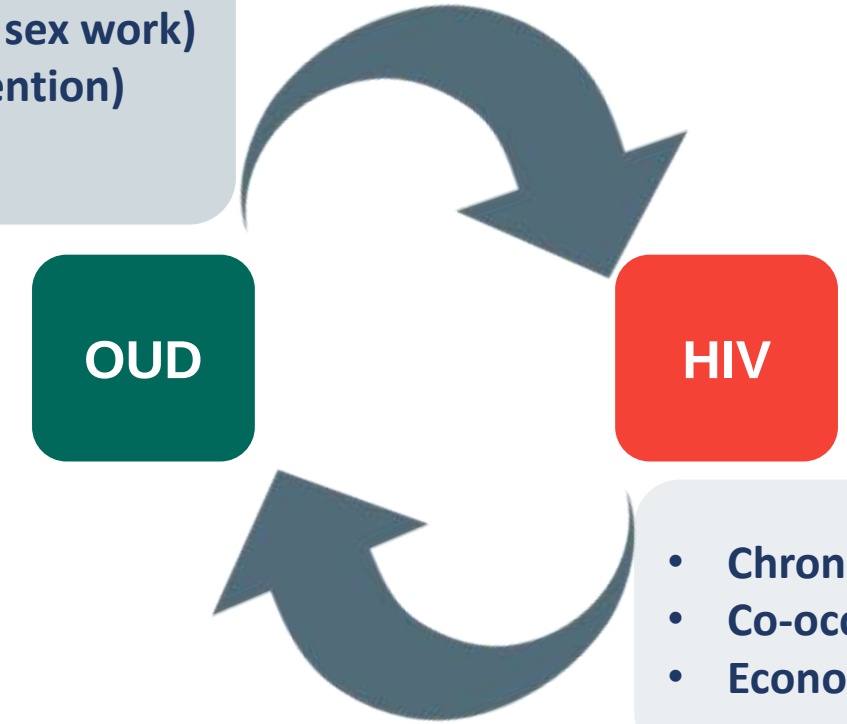
Summary

- Introduction
- Pilot study results
- Upcoming clinical studies: the AMOHI consortium
  - AMOHI 1: longitudinal RCT (outcomes)
  - AMOHI 2: cross-sectional cohort (pathogenesis)

Intersection of Opioid Use Disorders (OUD) and HIV infection

- *Metz V.E. Et Al. J Psychoactive Drugs. 2017 Jan-Mar;49(1):59-68*
- *Blackard J.T. Curr HIV Res. 2019 Jun 18 (ePub)*
- *Clayton H.B. Am J Prev Med. 2019 Aug 13. pii: S0749-3797(19)30270-3*
- *Brookmeyer K.A. Prev Med. 2019 Sep;126:105779*

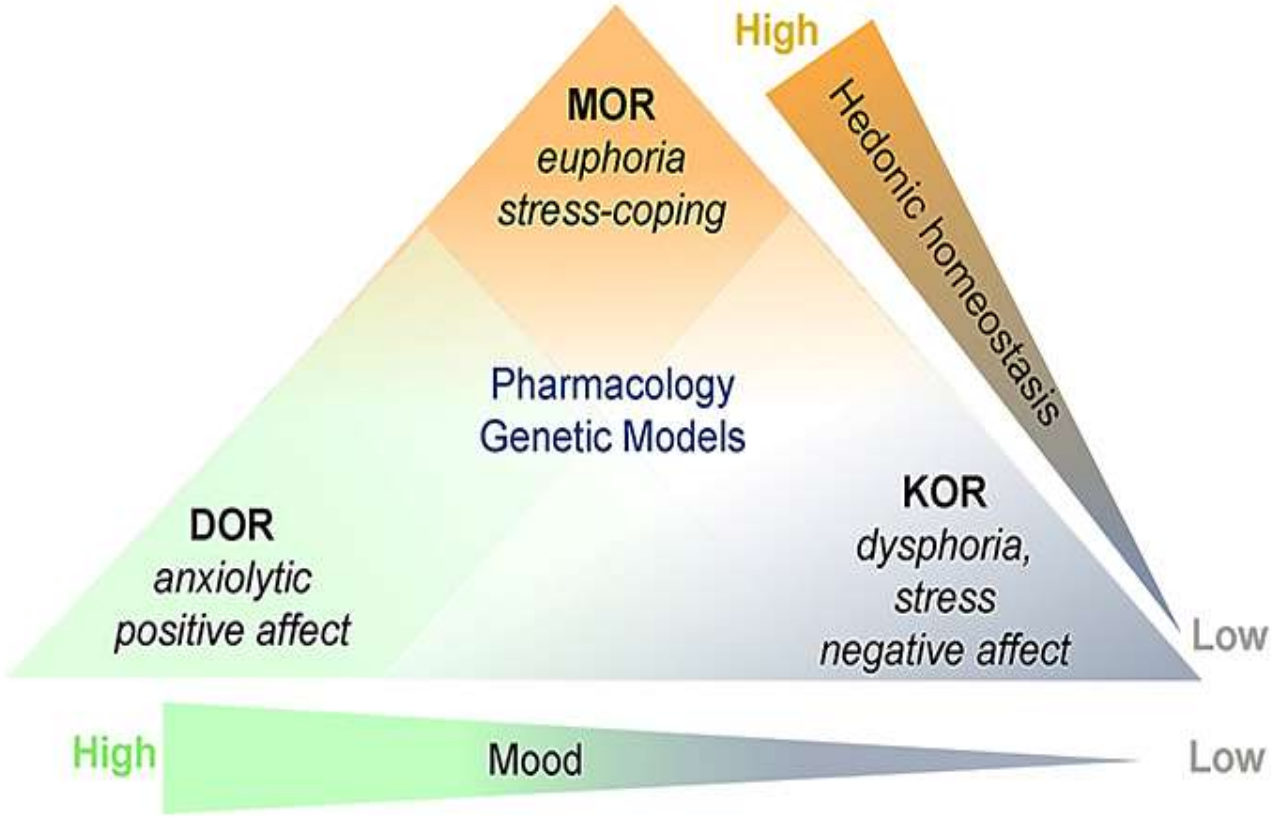
- **Increased exposure (needle sharing, sex work)**
- **Economic instability (access to prevention)**
- **Co-infections**



- **Chronic pain management**
- **Co-occurring mental health diagnoses**
- **Economic marginalization**

- *Cananc C et Al. Drug Alcohol Depend. 2019 Apr 1;197:141-148*
- *Carroll J.J. et Al. AIDS Behav. 2019 Apr;23(4):1057-1061*

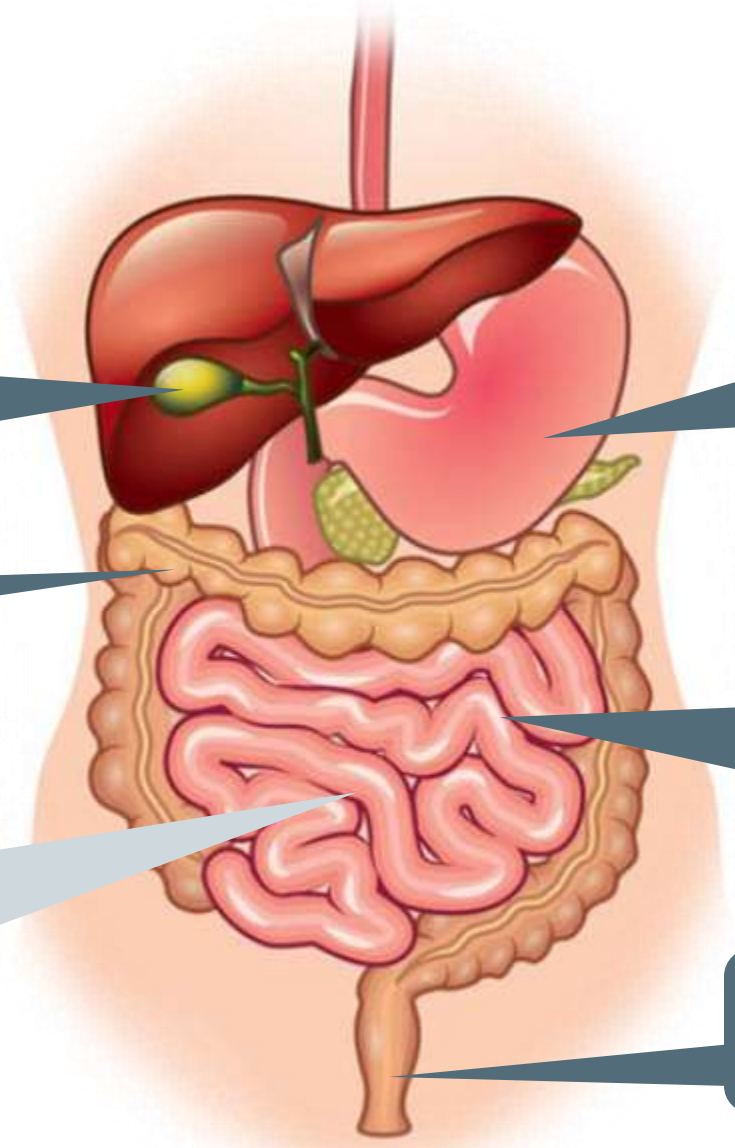
Background: opioid receptors



Receptor	Clinical effects	Location
μ	• Analgesia • Change in smooth muscle tone • Mood alteration • Nausea/vomiting	• Mesenteric plexus • Brain • Spinal chord • Sub-mucosal plexus
δ	• Decreases colonic transit time	• Mesenteric plexus • Brain
κ	• Central analgesia • Decreases colonic transit time • Visceral nociceptor antagonist	• Mesenteric plexus • Brain • Spinal chord • Sub-mucosal plexus

From Khansari et Al. Middle East J Dig Dis 5, 5-16, 2013

Background: Opioids and the GI tract



- ↓ gall bladder contraction
- ↑ stasis
- ↑ saturation and cholelithiasis

- ↓ motility
- ↓ secretion
- ↑ tone

- ↓ motility
- ↓ transit

- ↓ motility (ENS and CNS)
- ↓ peristaltic reflex
- ↑ tone
- ↓ secretion

- altered microbiome
- altered mucosal barrier
- microbial translocation

- ↑ tone
- ↑ sphincter sensory threshold

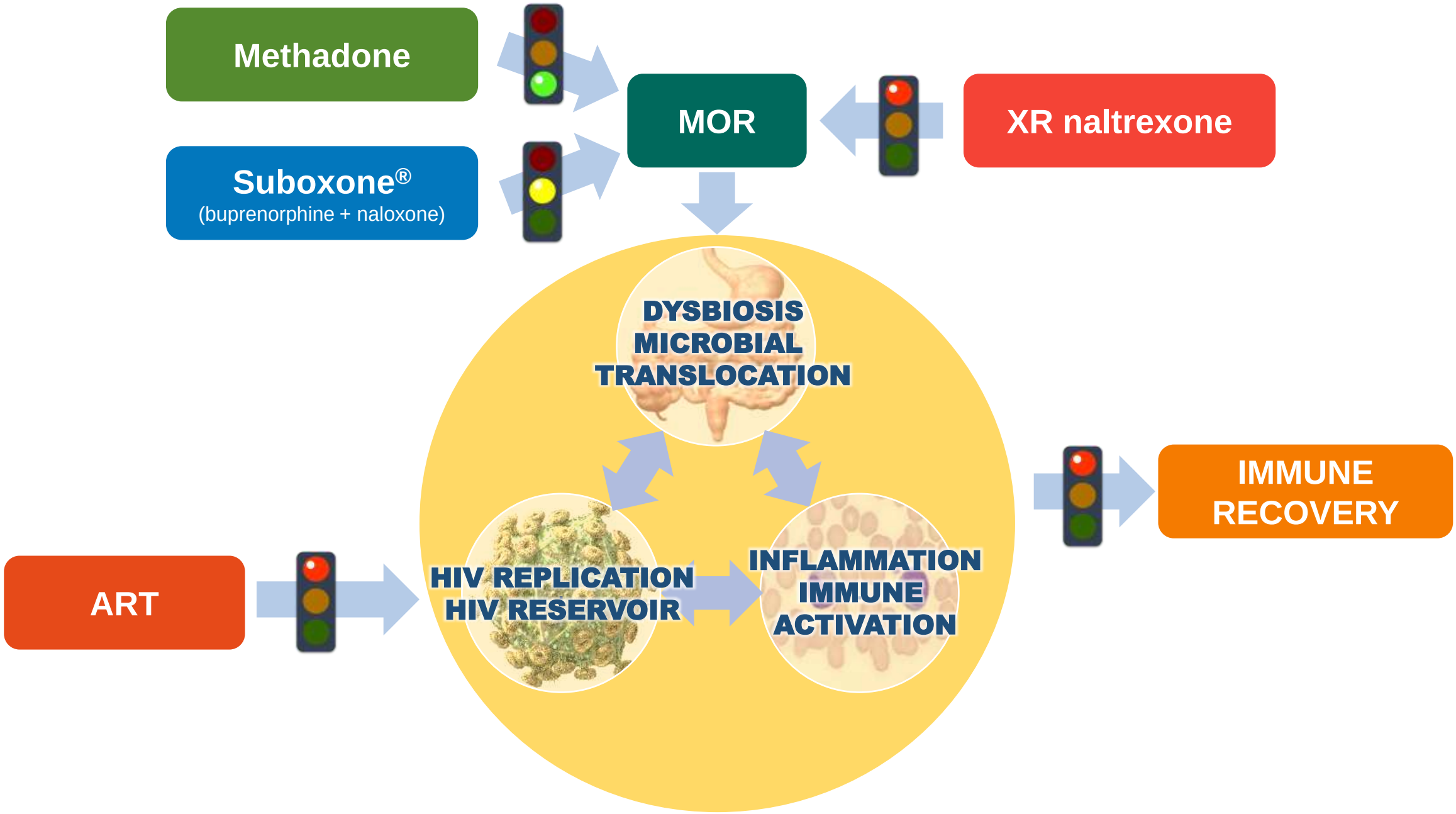
Sources: Khansari et Al. Middle East J Dig Dis 5, 5-16, 2013 | Banjeree et Al. Mucosal Imm 9, 1418-1428, 2016

Background: opioids and immune effectors

- Enhancement of cytokine production and T-cell proliferation (?)
- Modulation of cytokine and antibody responses
- Decrease in macrophage phagocytosis and chemotaxis
- Possible enhancement of HIV infection:
  - Increased HIV infection and replication in macrophages in vitro
  - Increased CREB-dependent HIV LTR-mediated transcription
  - Increased CCR5 and galectin-1 expression
  - Decreased antiviral factors (type-I IFNs, anti-HIV miRNAs)
  - MOR increases, KOR decreases infection in vitro

Sources: Khansari et Al. Middle East J Dig Dis 5, 5-16, 2013 | Jaureguiberry-Bravo et Al. Curr HIV Res 14, 417-430, 2017

Hypothesis: MOUD's potential effects on ART-mediated immune recovery



Working hypotheses

1. Proof-of-concept: HIV-infected, ART-suppressed individuals with OUD receiving a MOR antagonist will have lower immune activation than similar individuals receiving a MOR agonist

PILOT study (Cross-sectional proof-of concept)

Pilot study: Participant characteristics

STUDY GROUPS

Group	Treatment	Sex	AA	C/W	Other	Tot
MET	Methadone	F	1	4		13
		M	3	4	**1**	
NTX	XR-Naltrexone	F	2	1		14
		M	6	3	2	
Ctrl	Non-OUD	F		4		12
		M	5	2	1	

DEMOGRAPHICS and CLINICAL CHARACTERISTICS

Variable	Group	Mean	S.D.	Min	Max
Age	MET	50	11.9	25	62
	NTX	48	10.2	24	60
	CTRL	46	9.43	26	60
OST (Months)	MET	39	21.4	10.8	69
	NTX	19	9.7	8	37.3
	CTRL	n/a	n/a	n/a	n/a
ART (Yrs)	MET	12.5	9.4	0.8	29
	NTX	9.6	5.9	2	23
	CTRL	9.9	5.6	2	19
Nadir CD4	MET	186.2	151.2	7	588
	NTX	257.2	166.3	24	651
	CTRL	221.5	209.3	10	713
Current CD4	MET	605.8	430.1	132	1629
	NTX	550.8	278.6	252	1309
	CTRL	608	280.8	158	1240

Pilot study: sCD14 as a biomarker for HIV morbidity/mortality

Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection

Netanya G. Sandler,[1] Handan Wand,[10] Annelys Roque,[1] Matthew Law,[10] Martha C. Nason,[3] Daniel E. Nixon,[5] Court Pedersen,[8] Kiat Ruxrungtham,[9] Sharon R. Lewin,[11,12,13] Sean Emery,[10] James D. Neaton,[6] Jason M. Brenchley,[2] Steven G. Deeks,[7] Irini Sereti,[4] and Daniel C. Douek,[1] for the INSIGHT SMART Study Group

[1]Human Immunology Section, Vaccine Research Center, [2]Laboratory of Molecular Microbiology, [3]Biostatistics Research Branch, and [4]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [5]Department of Medicine, Virginia Commonwealth University, Richmond, Virginia; [6]Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis Minnesota; [7]HIV/AIDS Division, Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California; [8]Department of Infectious Diseases, Odense University Hospital, Odense, Denmark; [9]HIV Netherlands Australia Thailand Research Collaboration, Thai Red Cross AIDS Research Centre and Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [10]National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney; and [11]Infectious Diseases Unit, Alfred Hospital, [12]Department of Medicine, Monash University and [13]Centre for Virology, Burnet Institute, Melbourne, Australia

J Infect Dis. 2011 Mar 15;203(6):780-90

Do Biomarkers of Inflammation, Monocyte Activation, and Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People?

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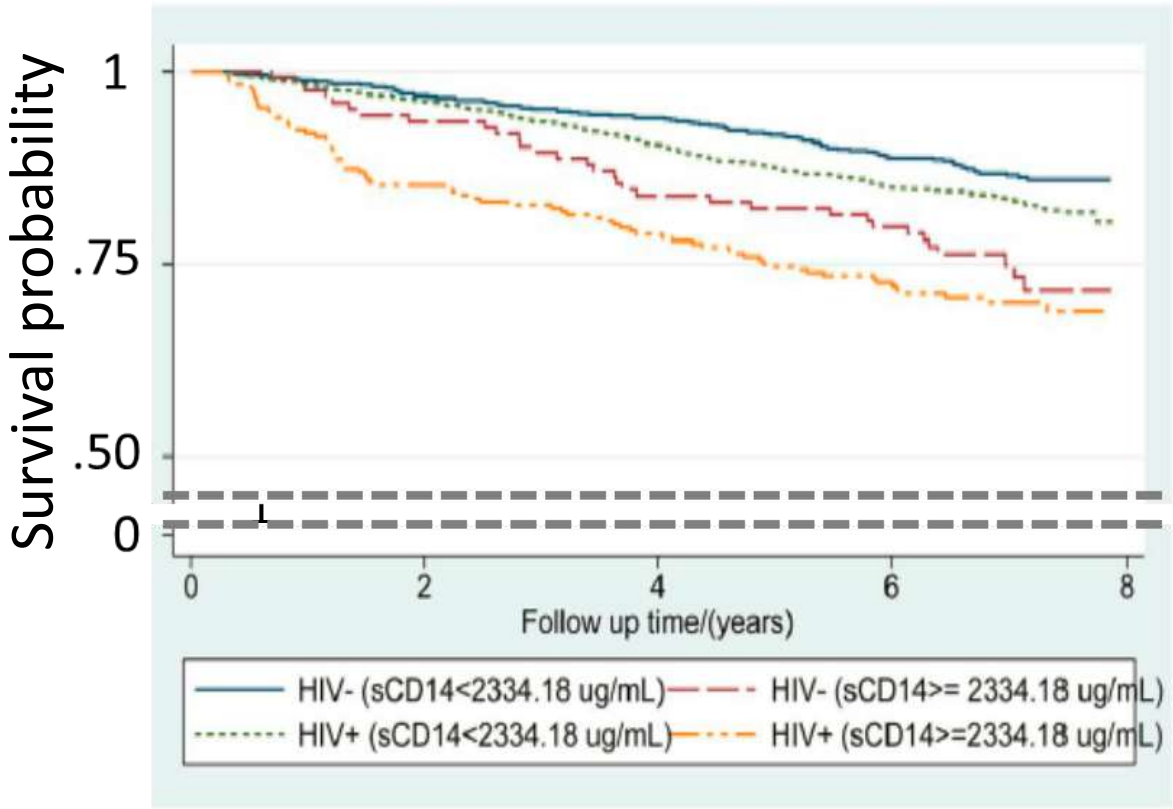
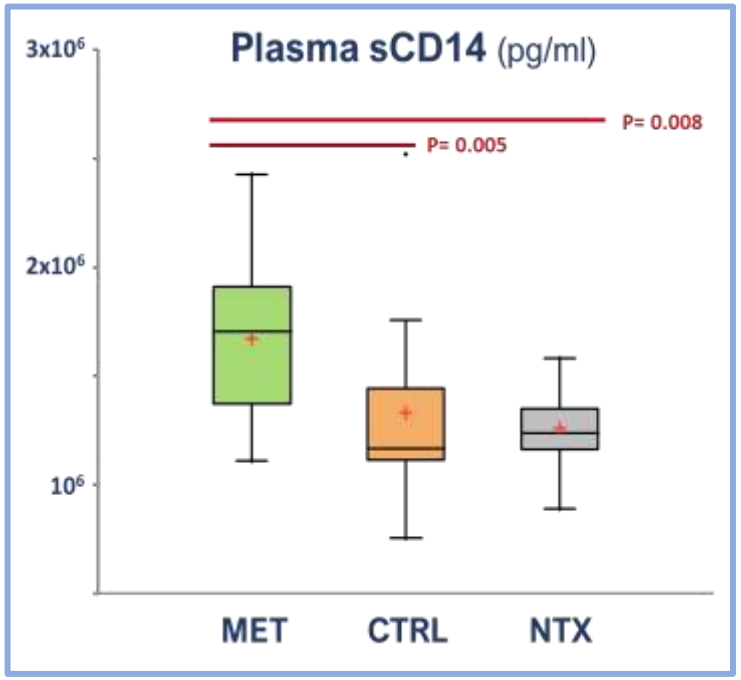
J Acquir Immune Defic Syndr. 2016 Jun 1;72(2):206-213.



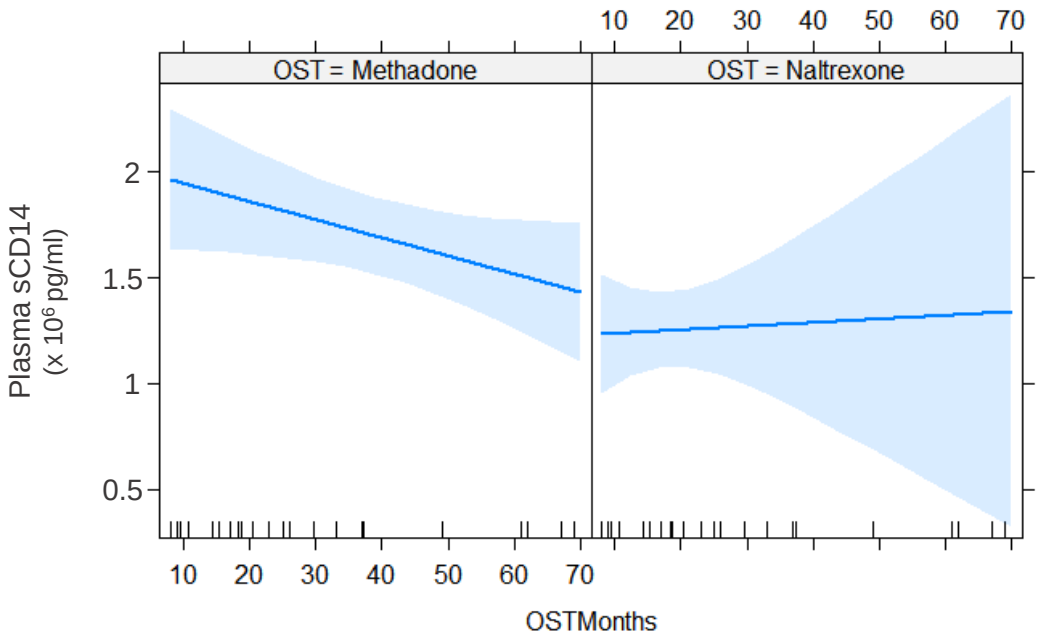
Pilot study: blood biomarkers of innate and adaptive immune activation

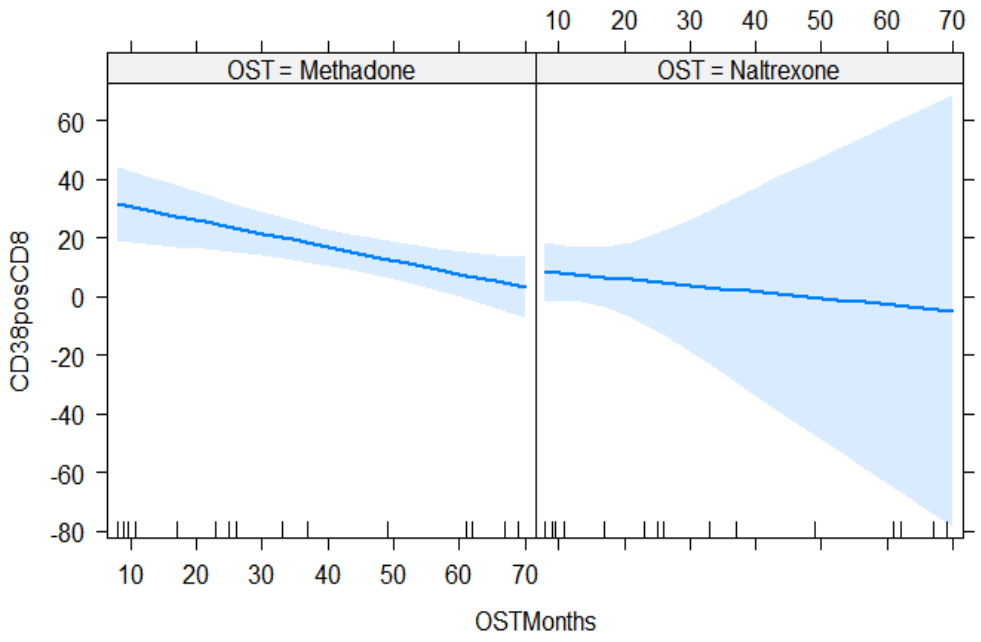
Marker	Group	Valid	Mean	Std. Dev.	ANOVA	CTRL Vs. MET	MET Vs. NTX	CTRL Vs. NTX
Plasma sCD14 *(x10^6 pg/ml)*	CTRL	14	1.33	0.4352	**0.015**	**0.005**	**0.008**	**ns**
	MET	13	1.672	0.3898				
	NTX	12	1.258	0.2036				
CD38$^+$ *(% of CD8+ T cells)*	CTRL	11	11.41	6.21	0.307	n/a	n/a	n/a
	MET	10	15.1	13.82				
	NTX	6	7.458	3.213				
CD38$^+$ HLA-DR$^+$ *(% of CD8+ T cells)*	CTRL	11	4.434	3.558	0.634	n/a	n/a	n/a
	MET	10	5.445	5.952				
	NTX	6	3.293	1.456				
CD163 MFI *(of total monocytes)*	CTRL	11	626.5	226.2	0.807	n/a	n/a	n/a
	MET	10	557.8	239.1				
	NTX	6	584.7	270.6				
CD169 MFI *(of total monocytes)*	CTRL	11	815.8	1627	0.274	n/a	n/a	n/a
	MET	10	137.8	78.8				
	NTX	6	129.1	62.51				
Cell-associated HIV DNA	CTRL	9	506.4	626.7	0.647	n/a	n/a	n/a
	MET	10	602.7	459.5				
	NTX	6	365.8	186.1				
Cell-associated HIV RNA	CTRL	12	702.8	910.1	0.291	n/a	n/a	n/a
	MET	13	1776	1761				
	NTX	12	1283	2112				



Pilot study: blood markers of innate and adaptive immune activation

EFFECT OF MAT REGIMEN AND DURATION ON sCD14 AND CD38⁺CD8⁺ T CELLS LEVELS





C38⁺ CD8 T cells	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	35.1885	6.9016	5.099	<0.0001
OST[T.Naltrexone]	-24.9361	10.8922	-2.289	**0.041**
OSTMonths	-0.459	0.1439	-3.189	**0.008**
OST[T.Naltrexone]:OSTMonths	0.2427	0.6058	0.401	0.696

sCD14	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2033704	188594	10.784	<0.0001
OST[T.Naltrexone]	-807557	273171	-2.956	**0.008**
OSTMonths	-8561	4251	-2.014	**0.058**
OST[T.Naltrexone]:OSTMonths	10267	10317	0.995	0.332

Pilot study: conclusions

- We compared two groups of ART-suppressed HIV-1-infected individuals with opioid use disorder and receiving long-term medication-assisted treatment with two medication with opposite MOR engagement:
  - Group 1: methadone (MOR agonist)
  - Group 2: XR-naltrexone (Vivitrol, MOR antagonist)
- Individuals receiving methadone had higher immune activation and microbial translocation than individuals receiving XR-naltrexone or control, non-OUD individuals, independent of time on treatment.
- Prospective studies are necessary to evaluate the clinical impact of MOR engagement on ART-mediated immune reconstitution.

Working hypotheses

1. Proof-of-concept: HIV-infected, ART-suppressed individuals with OUD receiving a MOR antagonist will have lower immune activation than similar individuals receiving a MOR agonist

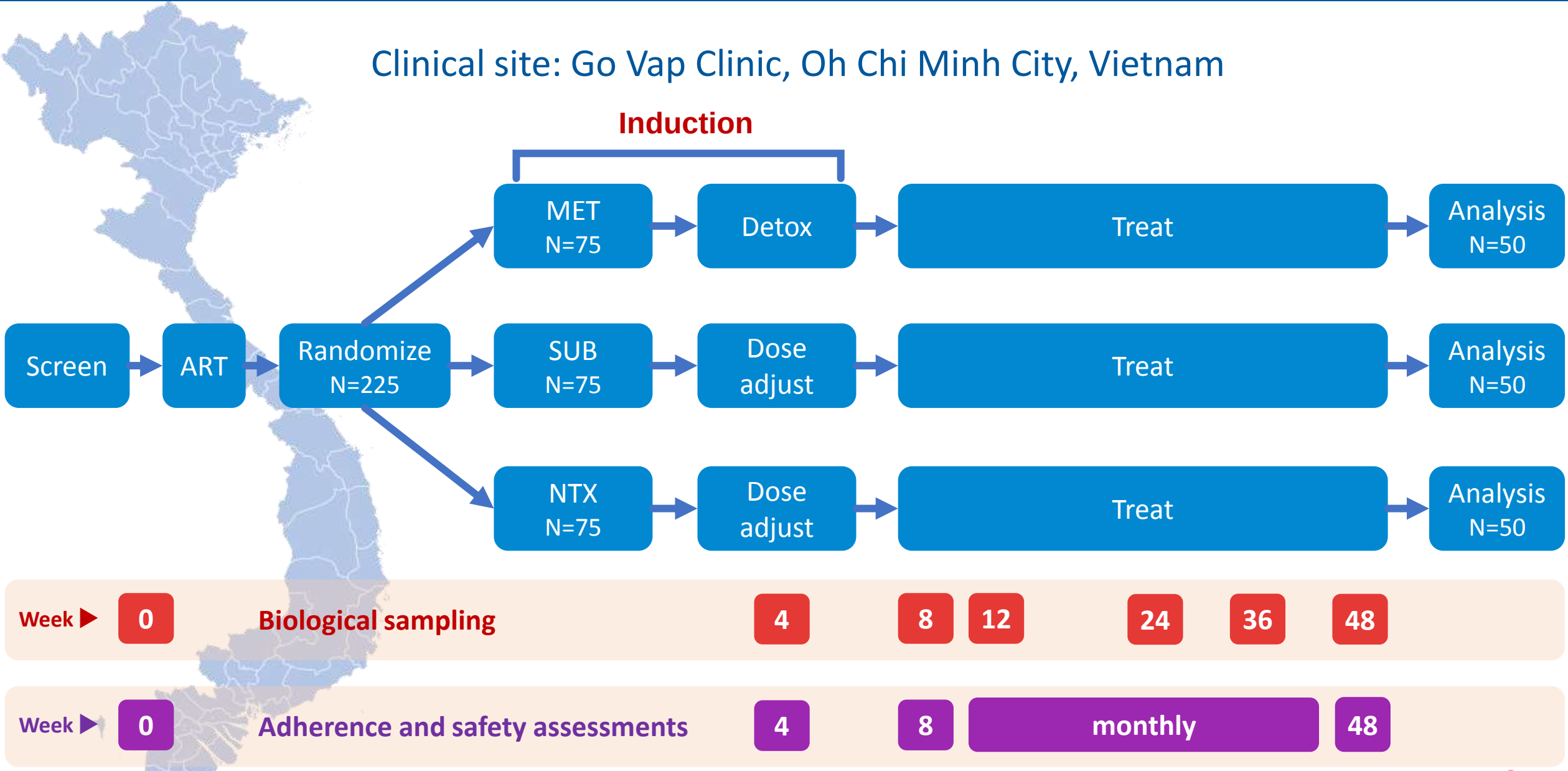
   PILOT study (Cross-sectional proof-of concept)

2. HIV-infected OUD individuals entering care will have better clinical, immunological and virological outcomes if treated with a combination of ART + MOR antagonists + Behavioral Drug and Risk Counseling (BDRC) than individuals treated with ART + MOR agonist + BDRC

   AMOHI project 1 study (Longitudinal RCT, Ho Chi Min City, Vietnam)

ART and MOUD Outcomes in HIV Infection (AMOHI 1) RCT | Summary

Clinical site: Go Vap Clinic, Oh Chi Minh City, Vietnam



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AMOHI 1 Study Clinical Interventions Summary

	Agent/test	Frequency	Notes
Assessments	• Addiction Severity Index • PHQ-9 • Urine drug screen test • Treatment Satisfaction questionnaire	• ASI/PHQ: weeks 0, 4, 12, 24, 48 • urine test/TSQ: monthly	
Medication	• methadone, Suboxone® or XR-naltrexone • cART	Daily or monthly D.O.T.	MAT as per randomization cART as per Government guidelines
Support	CBT-based Behavioral Drug and Risk Counseling (BDRC) (Denis C, Tran H, Nguyen L, Nguyen T, Trias V, Auriacombe M, Voisin A, Mai Thi Hoai S, Le Truong G, Daulouede JP, Metzger D, O'Brien C.P. NIDA International Conference; June 8-11, 2018; San Diego, CA, USA	Weekly for 3 months, monthly thereafter	• adherence to treatments • continued drug use and related drug and sex risk • cravings for drug use • psychological status (depression, anxiety, symptoms of psychiatric disorder) • confidence in and satisfaction with methadone or XR-NTX treatment • behavioral management strategies to be implemented until the next counseling session.

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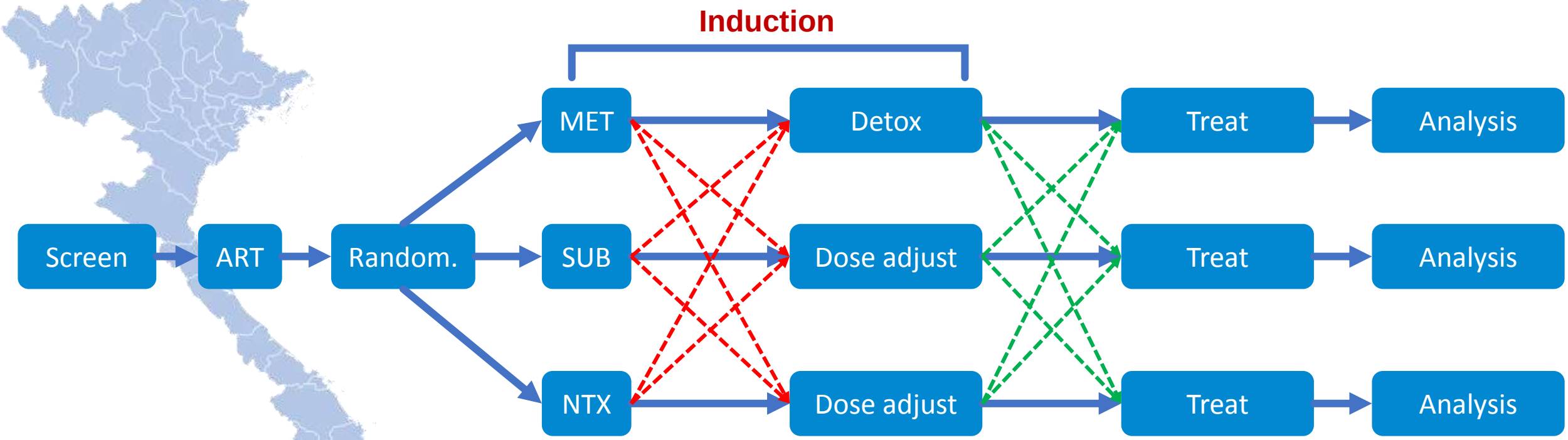
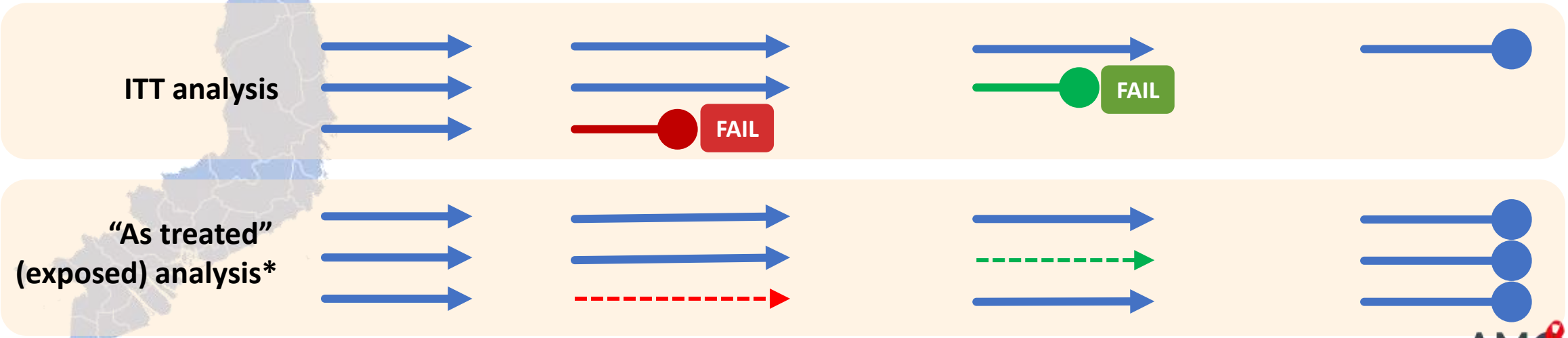
AMOHI 1 Analysis strategy





** Arm switching allowed only as an alternative to drop-out, and only within the first 5-8 weeks*

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AMOHI 1 Clinical and biological variables (1)

Category	Source	Principal variables
Clinical	n/a	• Retention in care • CD4 count • Continued drug use • Acceptability of MAT
Virology	Plasma	• Viral load suppression (<50 c/ml)
	PBMC	• Cell--associated DNA • RNA species (poly--A, multi--sliced Tat--Rev) • Transcriptional index

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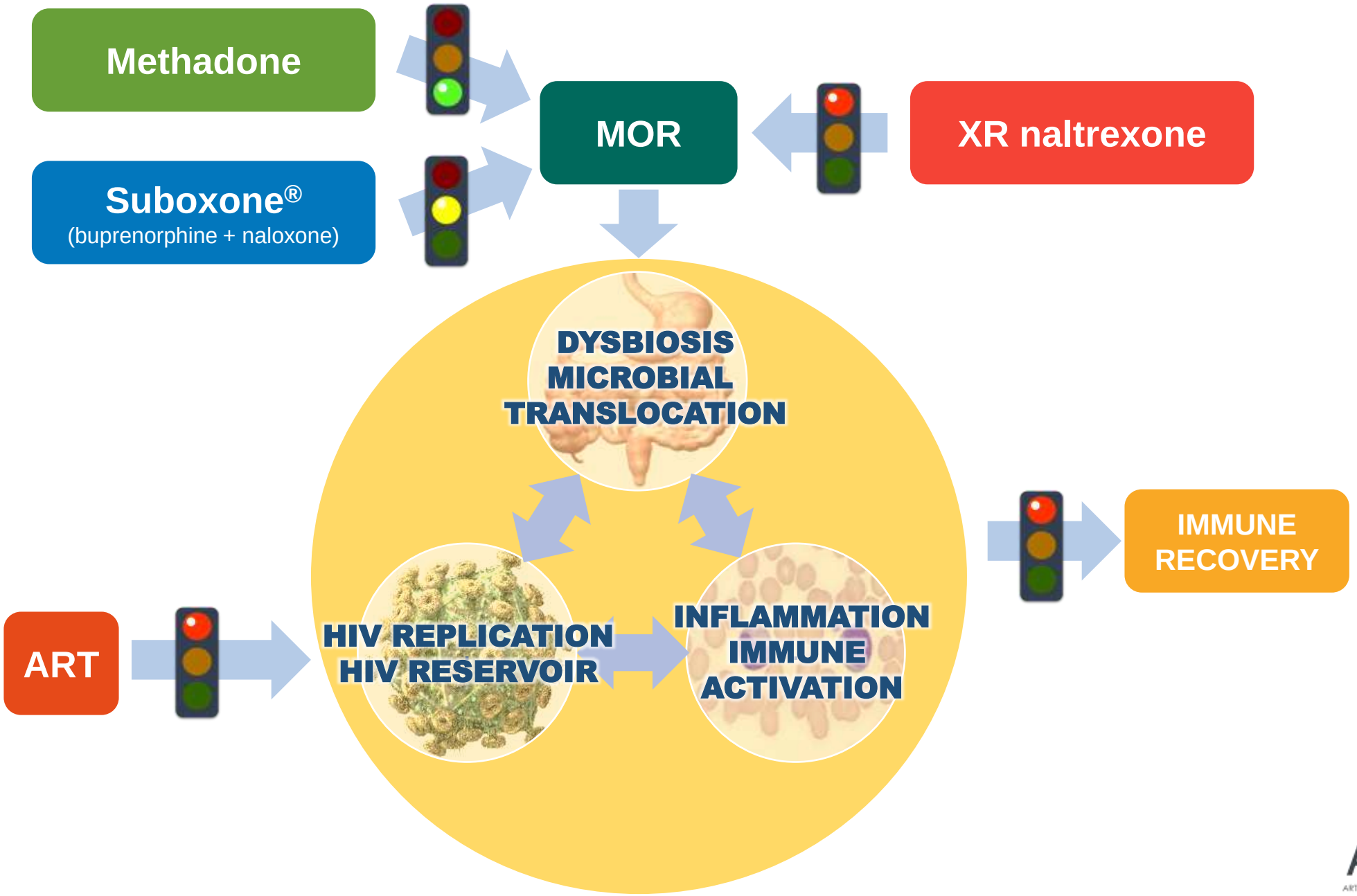
AMOHI 1 Biological variables (2)



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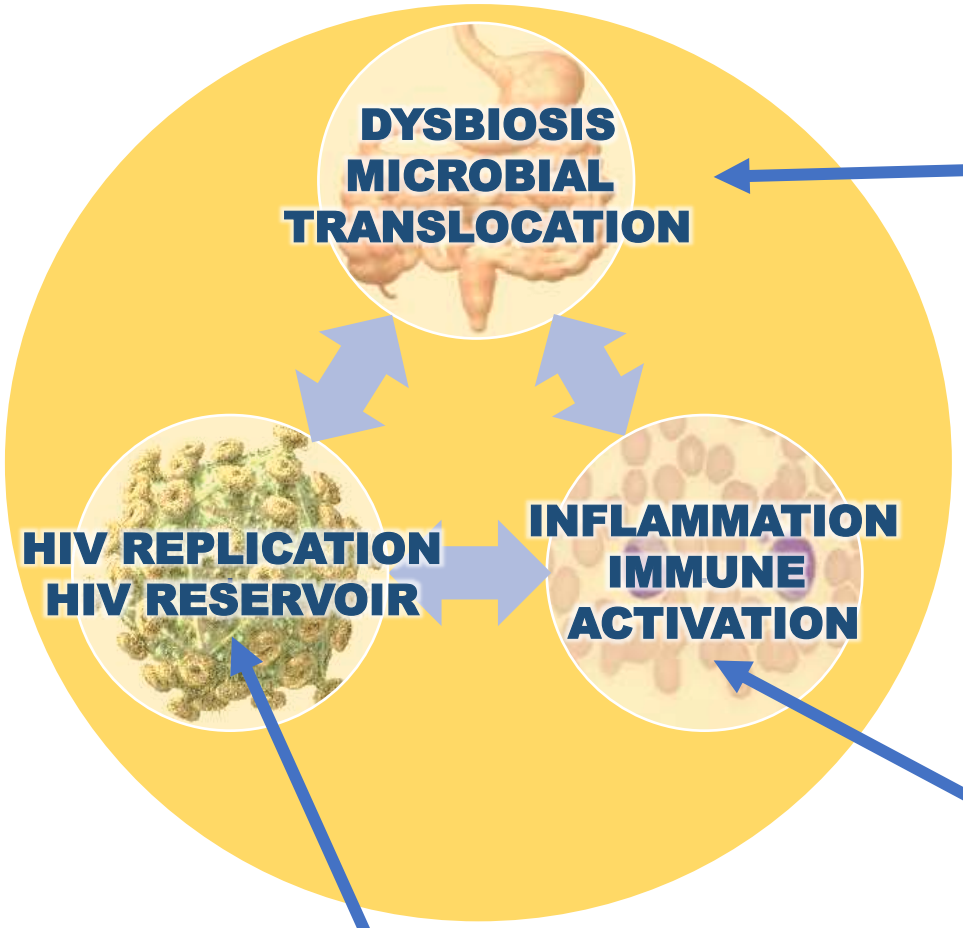
AMOHI 1 Biological variables (2)



- LBP, LPS, endo--CAB
- Intestinal fatty acid--binding protein (i--FABP)
- Cloudin, occludin, zonulin--1
- s16 rDNA
- Stool Butyryl--CoA (Inference on microbiome)
- *Cryopreserve stool for microbiomics analysis*

- CD38, HLA--DR and PD1 expression on CD8+ T cells
- CD169, CD14 CD16, MHCII, CD64 and CD206 on pro-inflammatory (intermed. $CD14^{+}/16^{+}$) monocytes
- Type--I IFN gene signature in monocytes
- hr--CRP and d--dimer
- sTNFR--1
- IL--6, IL--10, TGF--b
- sCD14 and sCD163 in plasma
- Plasma TNF-α, IL-1-β, IL-6, fractalkine/CX3CL1, SDF-1/CXCL12, CXCR4, CCL5/RANTES and CCR5, MCP1, MIP-1^{α}
- *NIDA: sCD27, YKL40, IP-10, sIL-1RII, sTNFR1/R2, IL-18BP, NFL, IL-6R, MCP-1/CCL2*

- Cell--associated DNA
- RNA species (poly--A, multi--spliced Tat--Rev)
- Transcriptional index

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Working hypotheses

1. Proof-of-concept: HIV-infected, ART-suppressed individuals with OUD receiving a MOR antagonist will have lower immune activation than similar individuals receiving a MOR agonist

   PILOT study (Cross-sectional proof-of concept)

2. HIV-infected OUD individuals entering care will have better clinical, immunological and virological outcomes if treated with a combination of ART + MOR antagonists + Behavioral Drug and Risk Counseling (BDRC) than individuals treated with ART + MOR agonist + BDRC

   AMOHI project 1 study (Longitudinal RCT, Ho Chi Min City, Vietnam)

3. In a HIV-infected, ART-suppressed individuals with OUD receiving a MOR agonist, a loss of mucosal integrity will be associated with increased microbial translocation, immune activation and latent viral reservoir levels in blood and GALT as compared to individuals receiving MOR antagonists

   AMOHI project 2 study (Cross-sectional mechanistic study, Philadelphia, PA USA)

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AMOHI 2 Study design

Cross-sectional, observational cohort study

Group	n	ART	OUD	MAT	MOR engagement
1	30	> 1 year	Yes	**Methadone** (daily oral)	Full agonist
2	30	> 1 year	Yes	**Suboxone®** (daily sublingual)	Partial agonist
3	30	> 1 year	Yes	**XR naltrexone** (monthly IM)	Full antagonist
4	30	> 1 year	No	**None** (HIV only)	None

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AMOHI 2 Sampling

	Visit 1	Visit 2, part A and B	
Test/Procedure	Screening	Blood sampling	Rectal mucosa biopsy
Clinical labs	X		
Questionnaires (ASI lite)	X		
Clinical history/updates	X	X	X
Targeted PE	X		
Drug screen [b] (urine)	X	X	
FibroScan	X		
275 ml Blood collection (Research)		X	
Sigmoidoscopy and rectal mucosa biopsy			X

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AMOHI 2 Biological variables, Part 1

Domain	Group	Source	Principal variables
Clinical	Immune recovery	Blood/other	• Nadir (or pretreatment) and current CD4 count • Continued drug use • Adherence to MAT • Viral load suppression (<50 c/ml)
Inflammation, activation and microbial translocation	Residual inflammation	Plasma	• hsCRP, d-dimer, MCP-1 and oxLDL
	Microbial translocation	Plasma	• LPS, LBP, endoCAb • sCD14 and sCD163 • 16S rDNA
	Immune activation	Plasma	• IL-1 beta, IL-6, IL-10, TGF-beta, sTNFR-1, and neopterin • sCD27, YKL40, IP-10, sIL-1RII, sTNFR1/R2, IL-18BP, NFL, IL-6R, MCP-1/CCL2
Gut mucosal integrity		Plasma	• Zonulin-1, iFABP, Cloudin, Occludin • Intestinal fatty acid--binding protein (i--FABP)

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AMOHI 2 Biological variables, Part 2

Domain	Group	Source	Principal variables
Immunophenotype (FACS/CyTOF)	T/NK cells	PBMC	• PD-1, CD69, CD38, HLA-DR on memory CD3+CD45RO+CD8+ T cells and CD56+CD16+ NK cells, NK (CD69, Perforin, CD57) • CXCR4, CCR5 on CD3+CD45RO+CD4+ T cells.
	Myeloid/DC	PBMC	• Activation of monocyte subsets(CD14+CD16-, CD14+CD16+, PDL1, CD169, CD40, CD86, • CD206) • co-receptor changes (CXCR4, CCR5).
HIV persistence	Cell-associated virus	PBMC	• Cell/associated DNA and RNA in $CD45^{+}CD3^{+}CD4^{+}$ T cells
	Inducible virus	PBMC	• P24 SIMOA
MOR/TLR interplay	TLR 2/4 responses	PBMC	• HIV coreceptors (CCR5), TLR4 expression, and intracellular proinflammatory cytokine expression (TNF-alpha, IL-1 beta)
Tissue analysis	Immune activation	GALT cells	• CyTOF analysis (see next slide)
	TLR and type-I IFN signatures	GALT cells	• RNA-Seq analysis

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AMOHI 2 Biological variables: flow mass cytometry (CyTOF) antibody panel

Parent cell	Subsets	Function	
CD3+ T cells	CD4 CD8 CD45RO CD45RA CCR7 CD27 Siglec-1 CD11b CD68 CD127 CD1c CD370 CD123 CD16 CD56 CXCR4 CCR5 CD57 NKp46 NKG2A NKG2C	CD38 HLA-DR PD1 CD69 CD25	Activation/ Exhaustion
CD19+ B cells			
CD3-CD7+ innate cells		CCR4 CXCR3 CCR6 β7 CD103	Homing/ Migration
CD14+ monocytes			
CD33+CD68+ macrophages		Tetherin MX-1 IFN-aR1 ISG-15 USP-18 IFN-γ IFN-α IFN-β	IFN-associated activation
CD11c+ dendritic cells			
CD66a+ granulocytes			

CD45+ hematopoietic cells

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AMOHI 1

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