NCG GUIDELINES- 2019

DRAFT GUIDELINES

ADULT HEMATOLYMPHOID MALIGNANCIES

Contents

Applicability of the guidelines

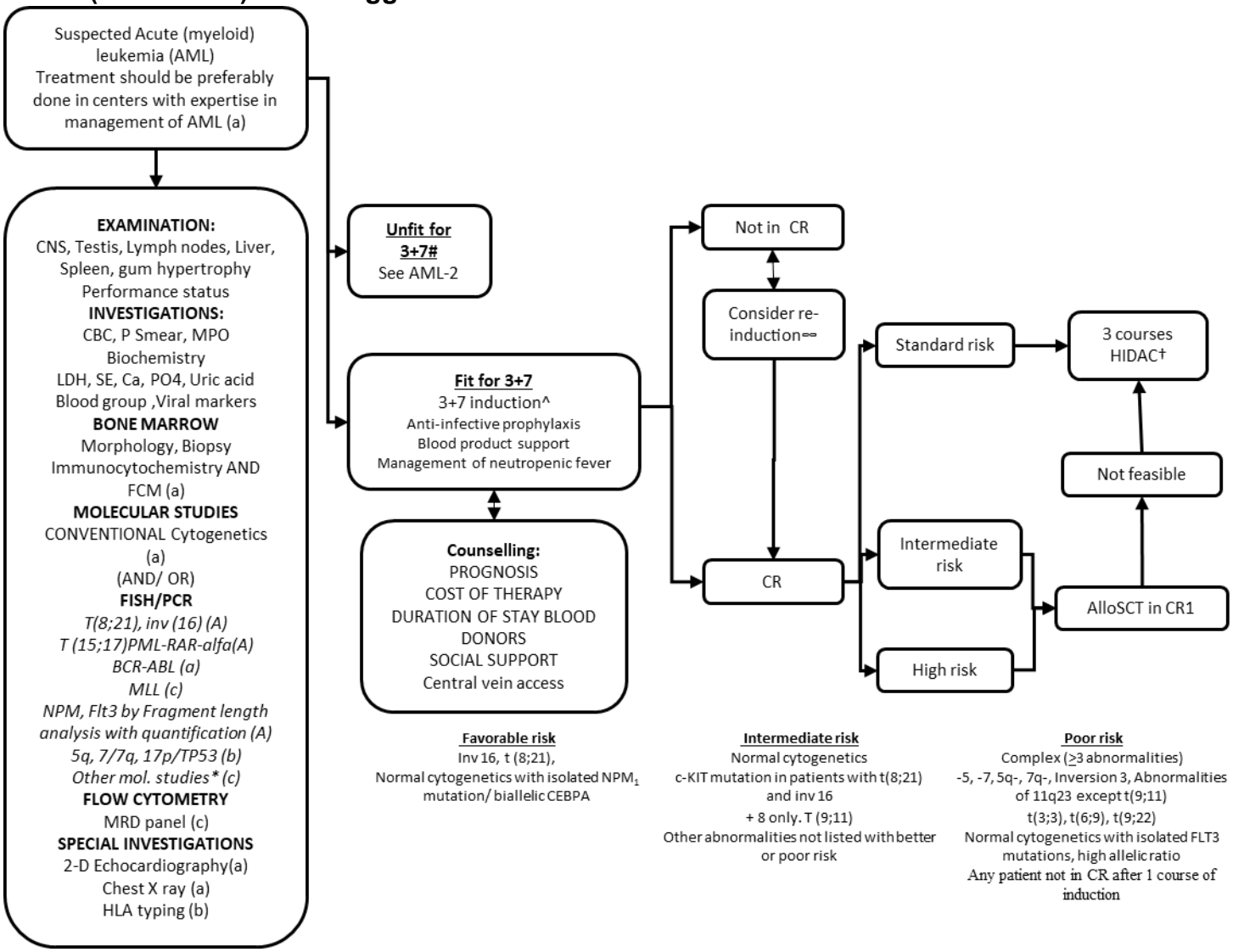
a. Essential:

b. Optimal

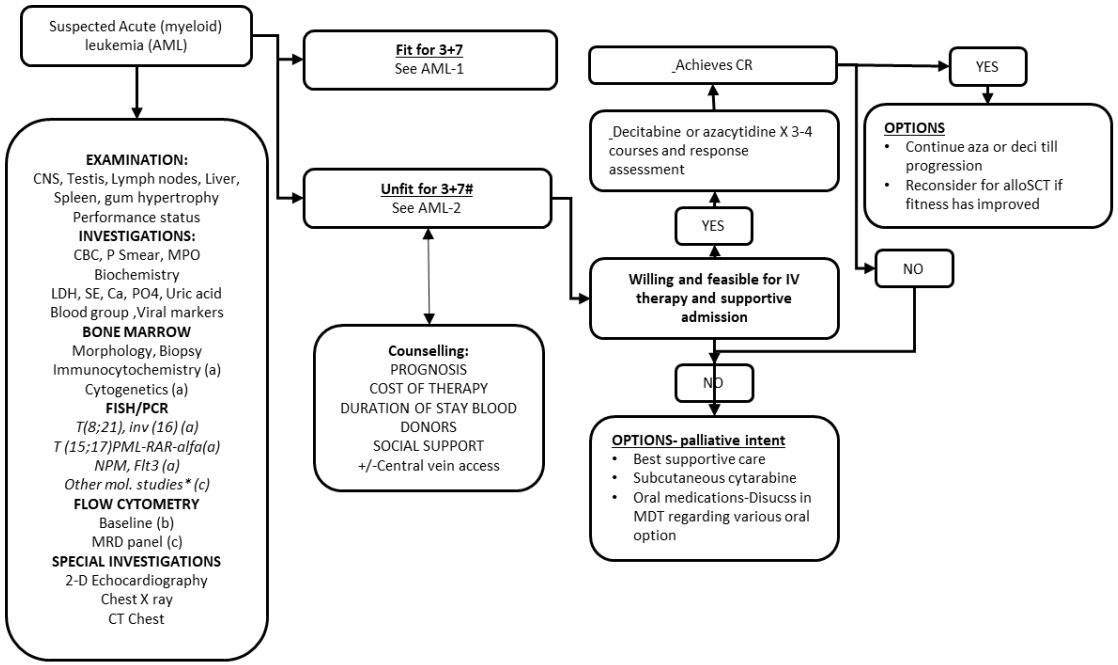
c. Optional

Acute Myeloid Leukemia

AML-1 (non APML)- Fit for aggressive Rx



AML-2 (non-APML) Unfit for aggressive Rx

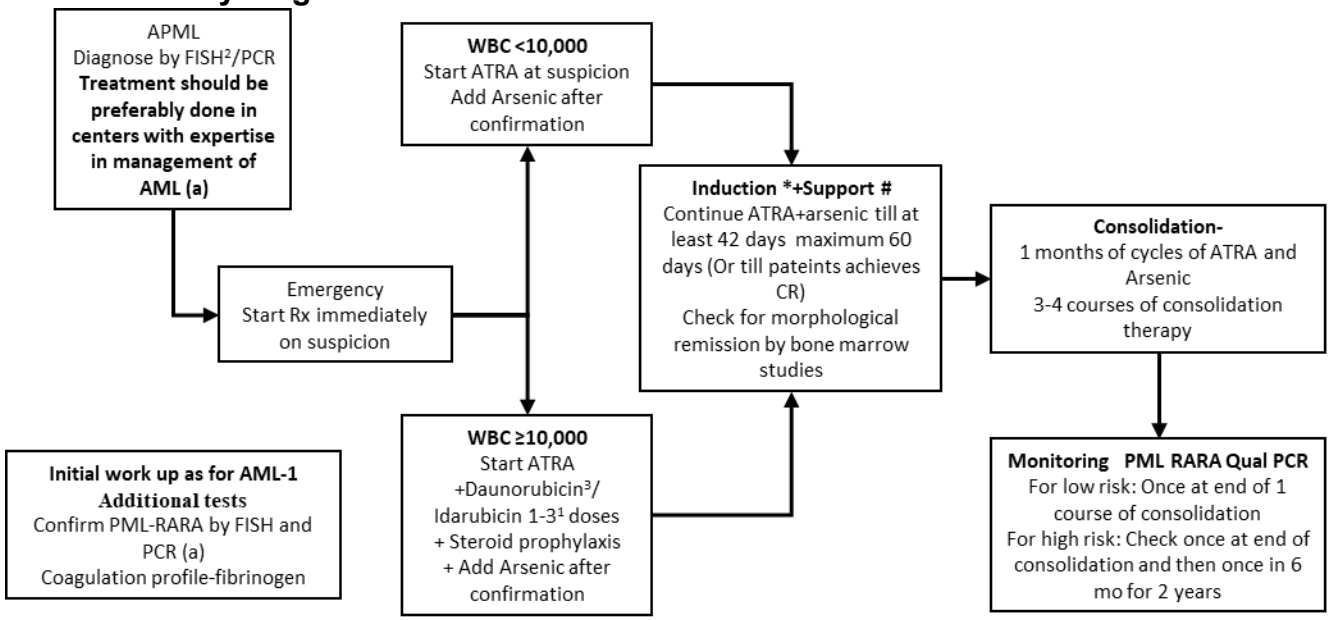


*Other molecular studies *CEBP alfa, kit mutations, IDH mutation*

Fitness for intensive therapy 3+7 in AML is a complex clinical decision using the following parameters: age, performance status, baseline infection, comorbidities, patient willingness, individual institutional protocols.

Acute Promyelocytic Leukemia

APML-1- Newly diagnosed



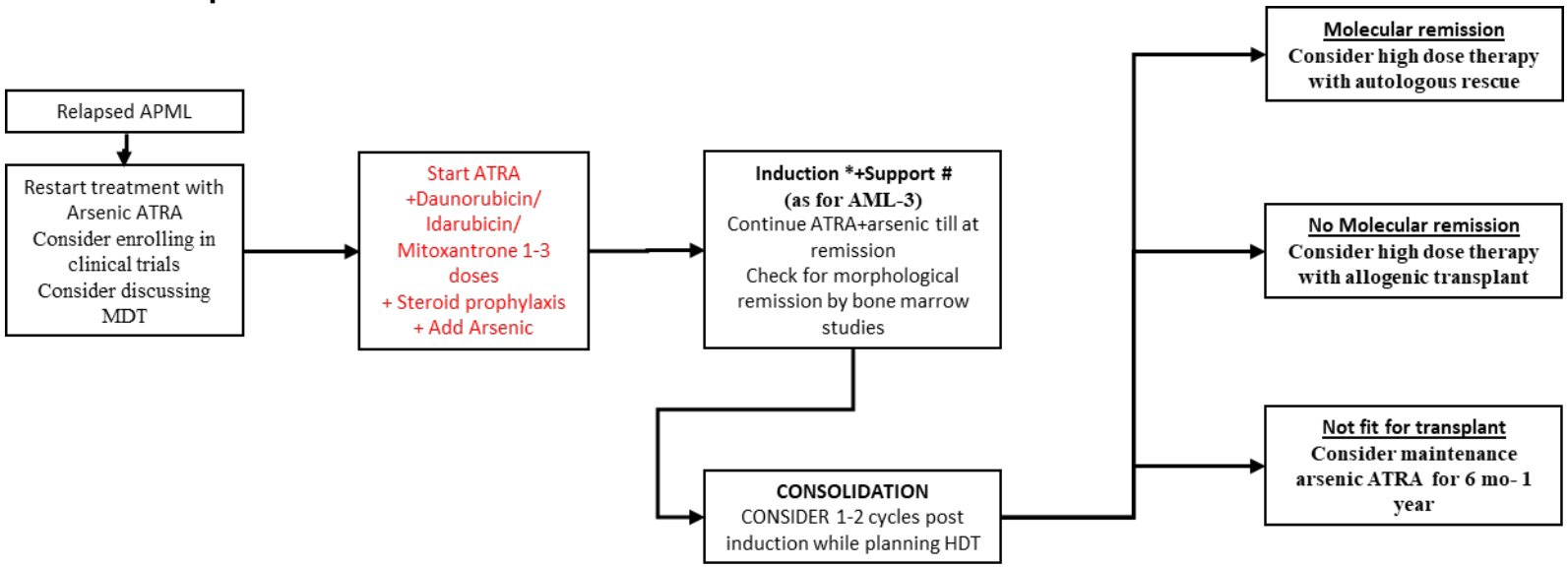
#Supportive care

- Platelet transfusion to keep platelets > 50,000/cmm
- FFP or cryoprecipitate transfusion to keep fibrinogen >150mg/dL

*** Management of Sudden increase in WBC/ differentiation syndrome (DS)**

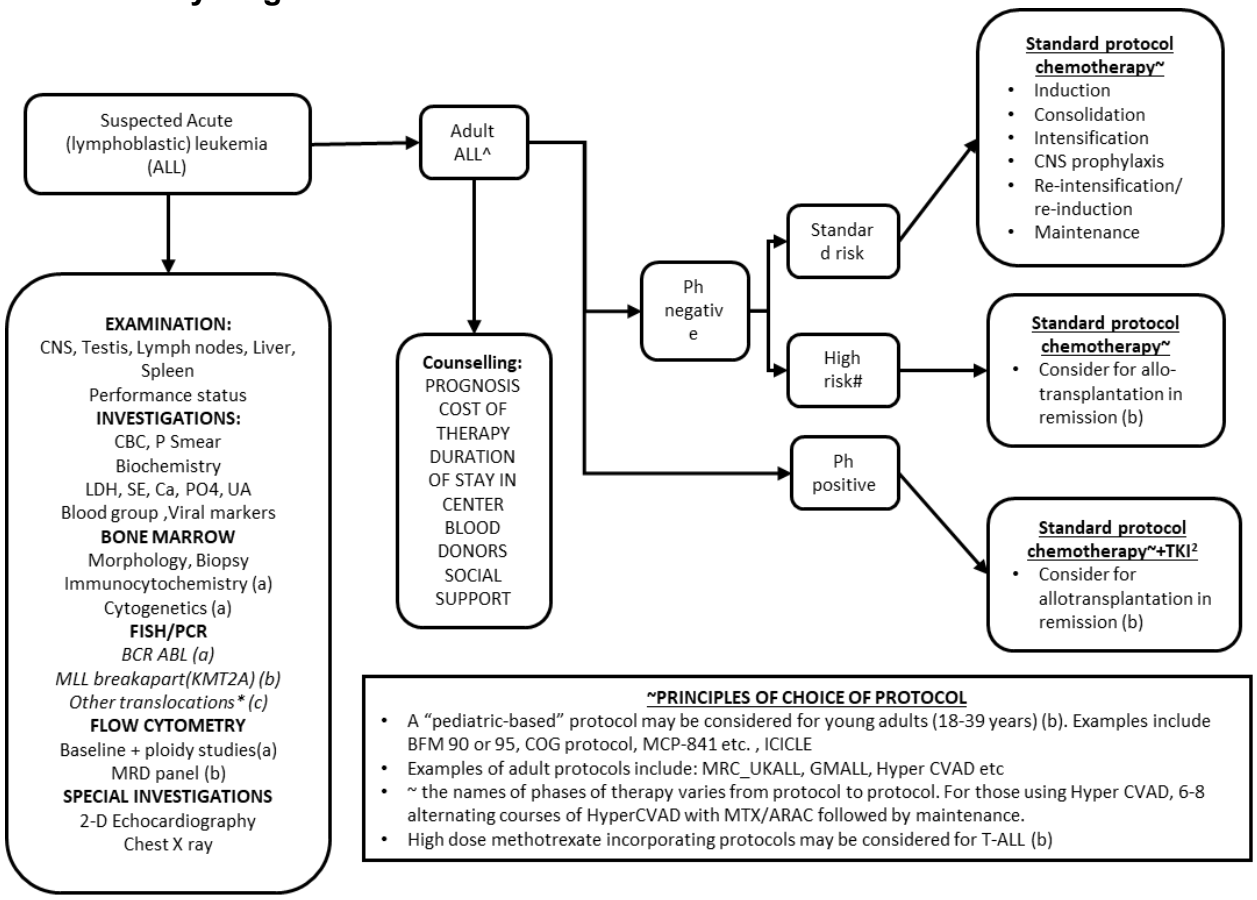
- Start Hydroxyurea/ Daunorubicin for controlling counts
- Steroids if not started already- dexa 8 mg BD
- For severe DS, hold ATRA/ Arsenic temporarily
- Management for febrile neutropenia as per high risk guidelines
- 1. Addition of anthracyclines based on fitness and after confirmation
- 2. Ideally breakapart probe to be used to identify variant translocations
- 3. To check whether anthracyclines required in high risk APML or options

APML-2- Relapsed APML



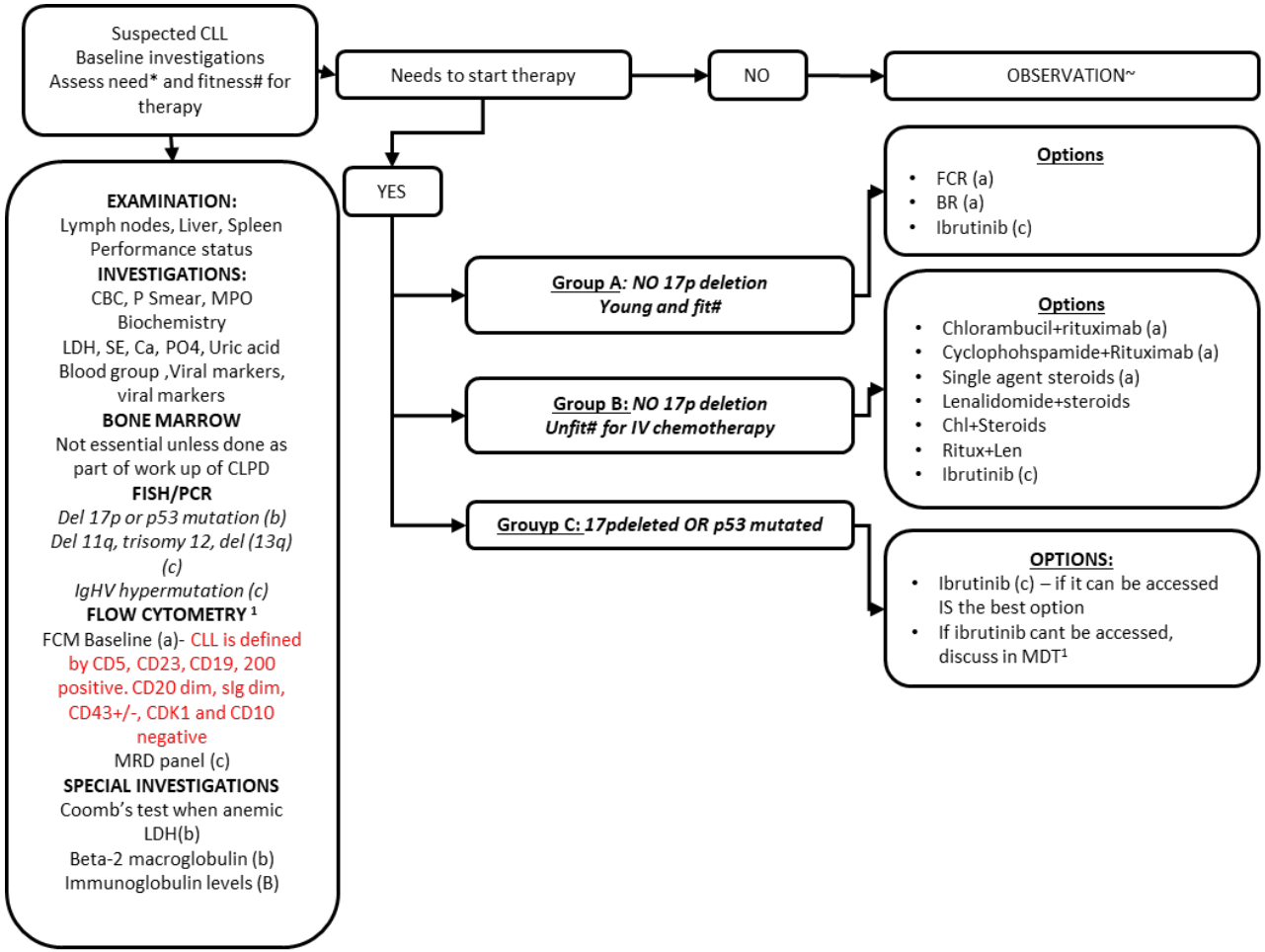
Acute Lymphoblastic Leukemia

ALL-1- Newly diagnosed ALL



* Other molecular studies *ETV6-RUNX1, TCF3-PBX1 (1;19), Ph-like abnormalities, iAMP21, ETP-ALL, 3 centromeric probes for 7 and 10*
^ MPAL (mixed phenotype ALL) is usually treated like a high risk adult ALL
# High risk: Any of the following: Ph or BCR-ABL positive, MLL rearrangements, No day 8 blast clearance, MRD positivity, ETP-ALL, Ph –like genotype

Chronic Lymphoid Leukemia



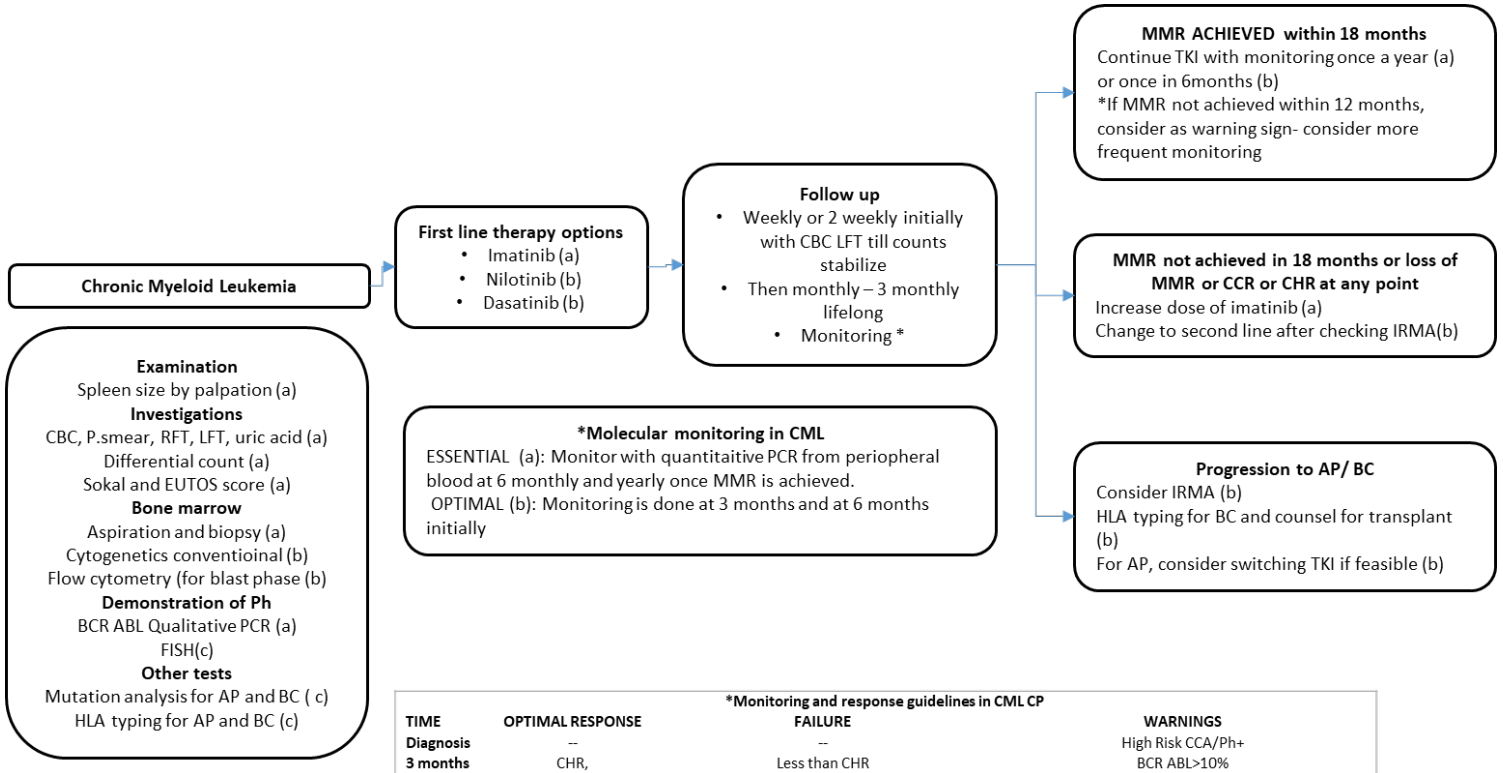
*NEED for therapy in CLL: B symptoms/end organ dysfunction/progressive bulky disease (spleen.6cm, LNs >10cm) anemia/thrombocytopenia, lymphocyte doubling in less than 6 months

#FITNESS for therapy in CLL: age>70 years, comorbidities, performance status 2 or more

~OBSERVATION in CLL: Review once in 3 months with physical examination, history and blood counts, start therapy if there is indication

Chronic myeloid leukemia

CML-1-chronic phase



*Monitoring and response guidelines in CML CP

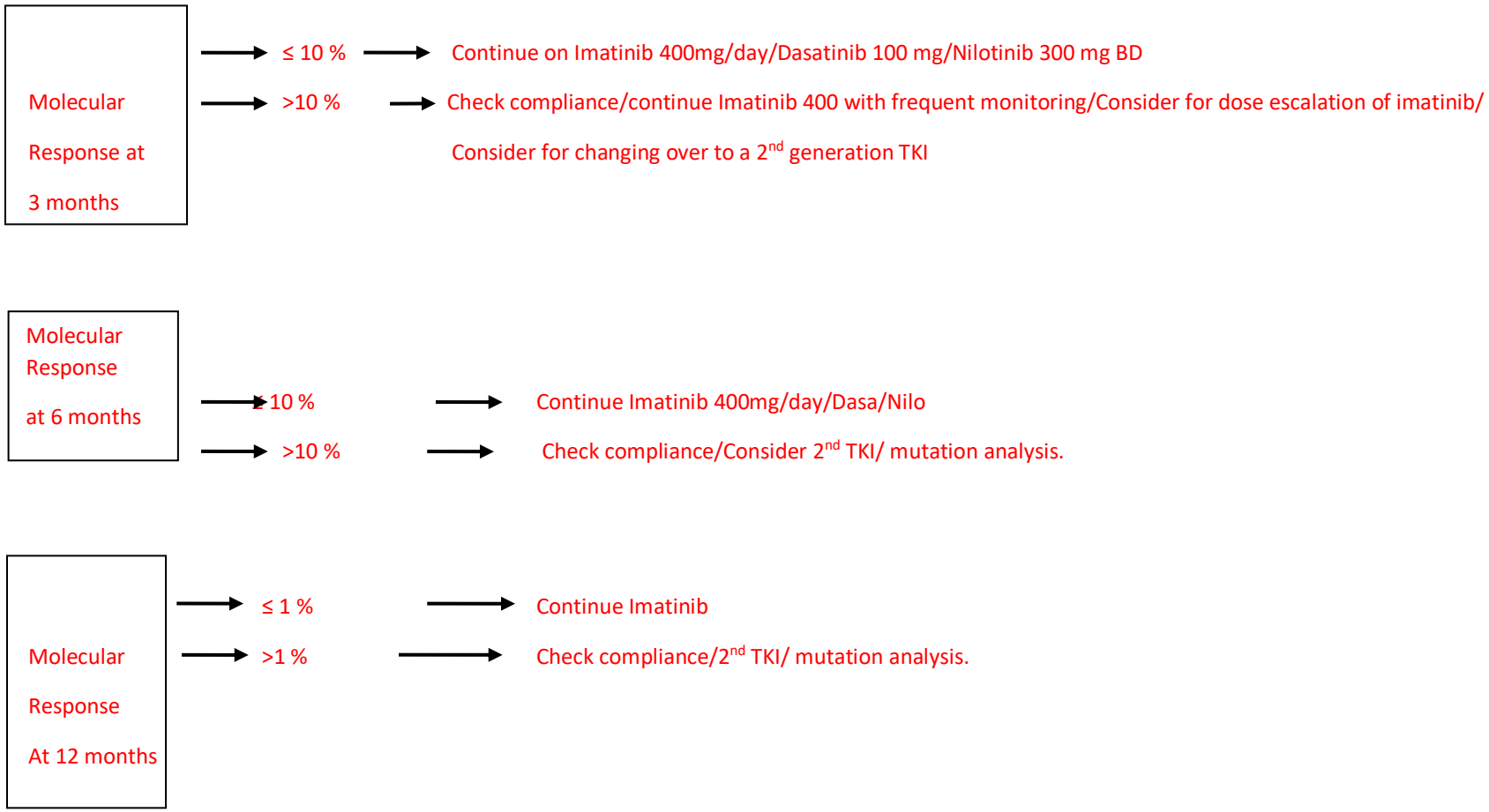
TIME	OPTIMAL RESPONSE	FAILURE	WARNINGS
Diagnosis	--	--	High Risk CCA/Ph+
3 months	CHR, BCR ABL<=10% P CyR	Less than CHR Ph>95%	BCR ABL>10% Ph>36-95 %
6 months	BCR ABL<=1% C CyR	BCR ABL>10% Ph>35%	BCR ABL 1-10% Ph 1-35%
12 months	BCR ABL<=0.1%	BCR ABL>1% Ph>0%	BCR ABL 0.1 - 1%
Any time	MMR or better	Loss of CHR, Loss of CCgR, Mutations, CCA/ Ph+	Increase in transcript levels /CCA/ Ph+(-7/7q-)

Optimal Response: No indication that a change of therapy may improve a survival that is currently projected as close to 100% after 6 to 7 years.

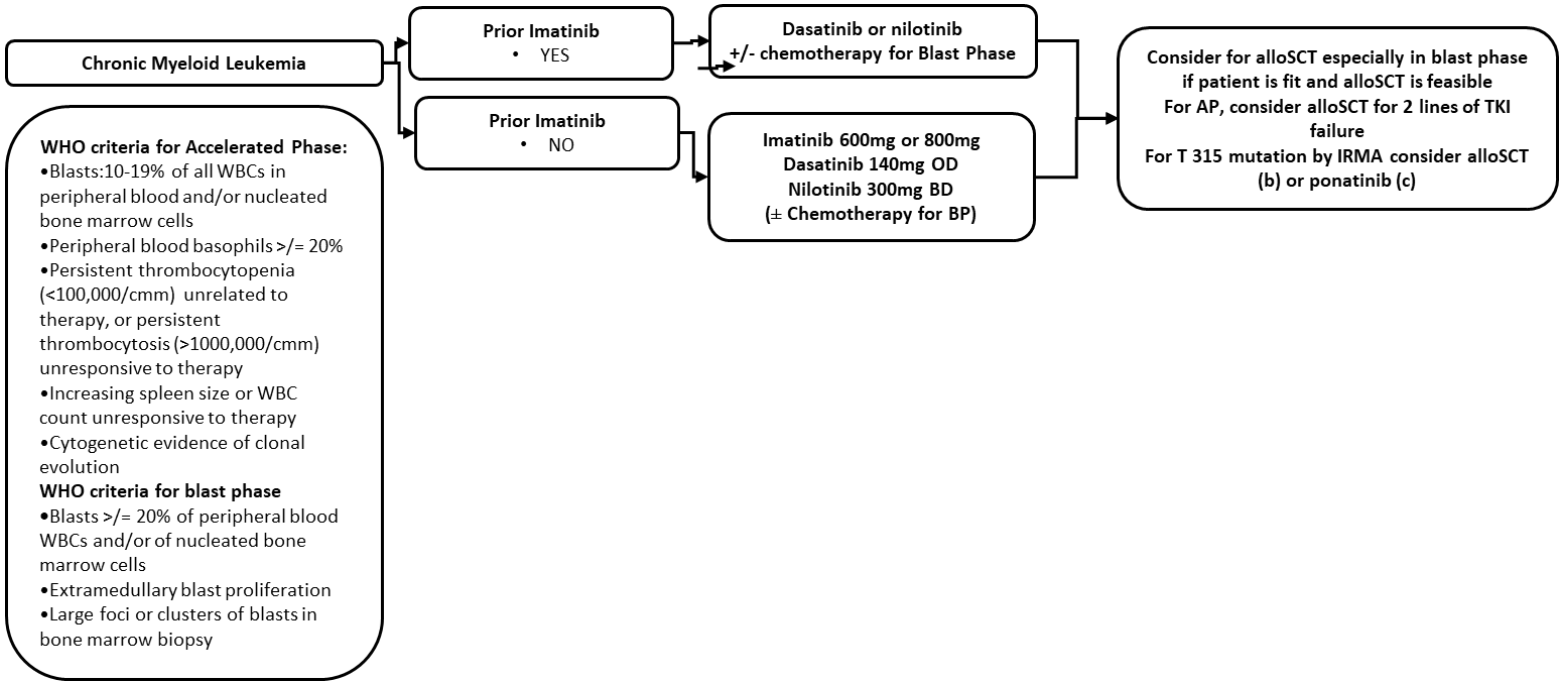
Failure: Favourable outcome is unlikely, should receive a different treatment, whenever available and applicable.

Warning: May require a more stringent and careful monitoring.

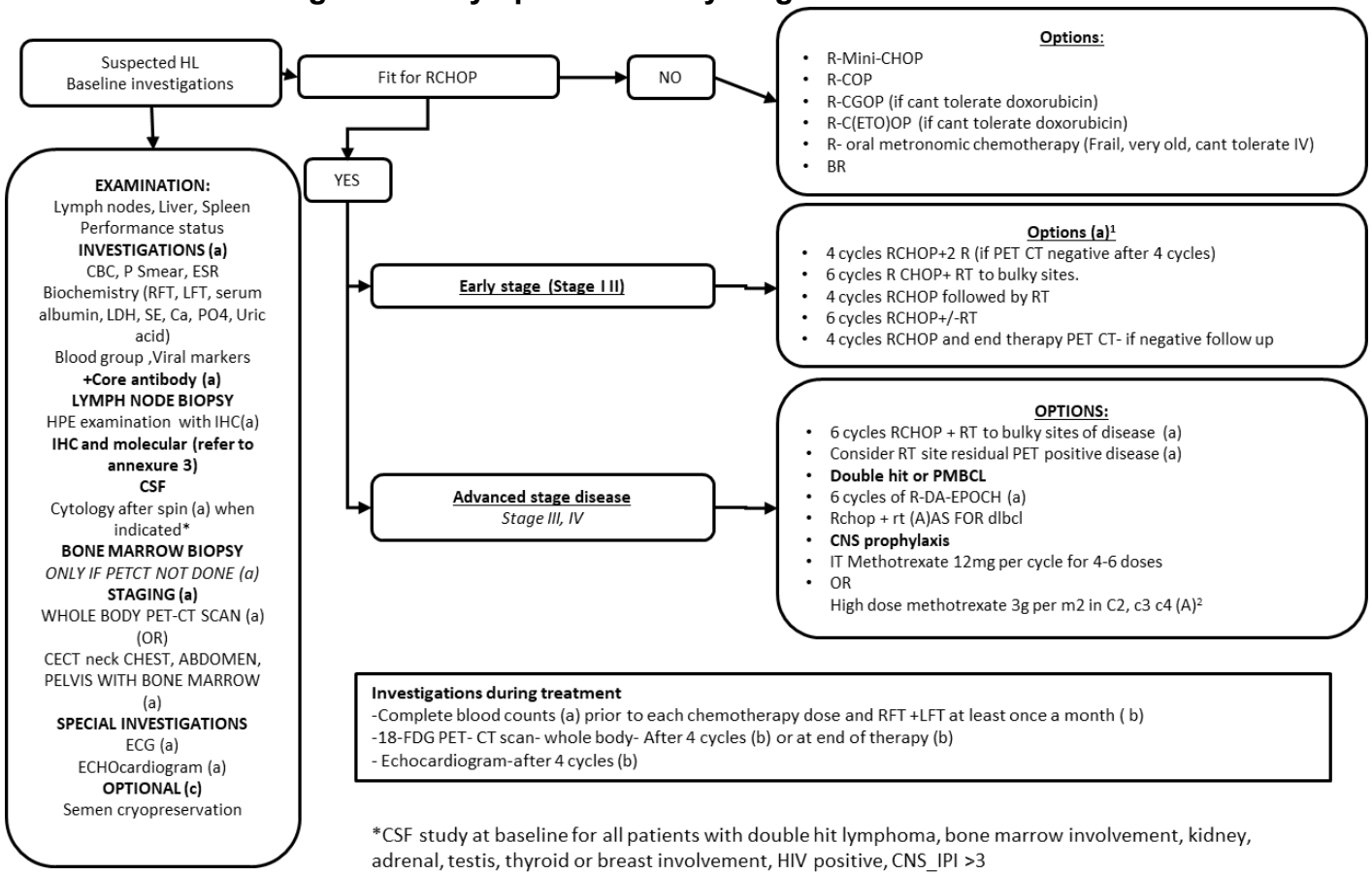
CML-2- Practical Management algorithm for CML based on Molecular response



CML-3- CML accelerated and blast phases



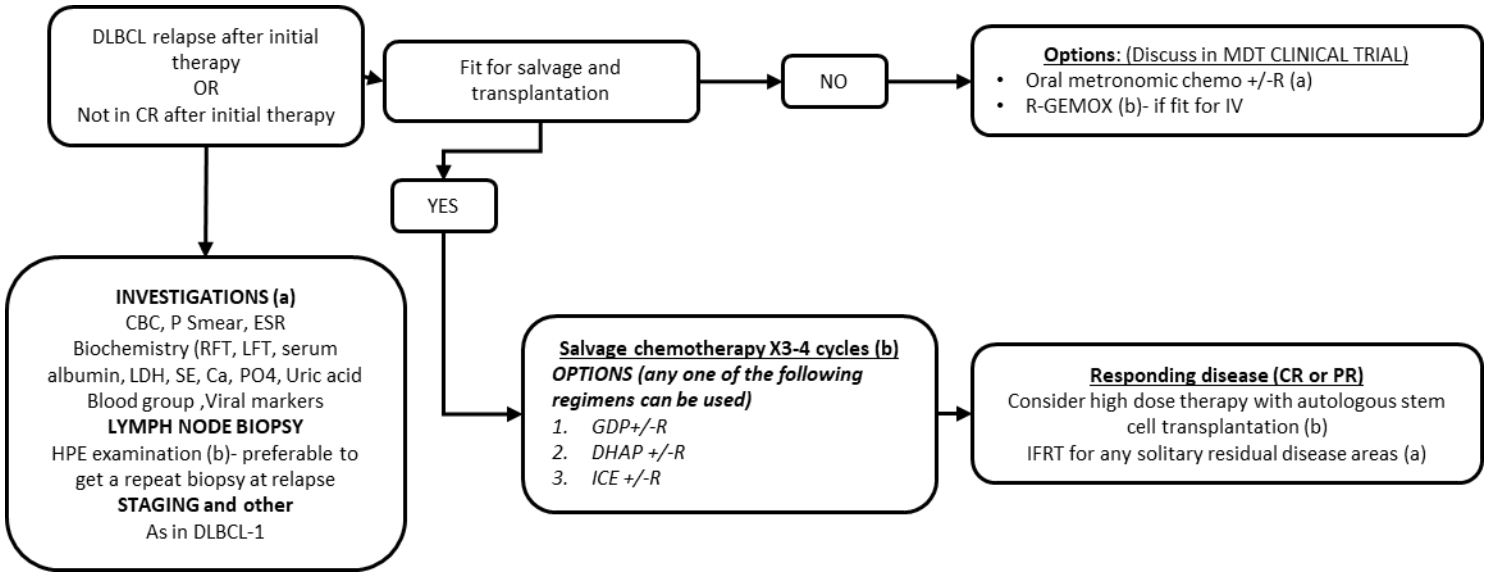
DLBCL-1 Diffuse Large B Cell Lymphoma- newly diagnosed



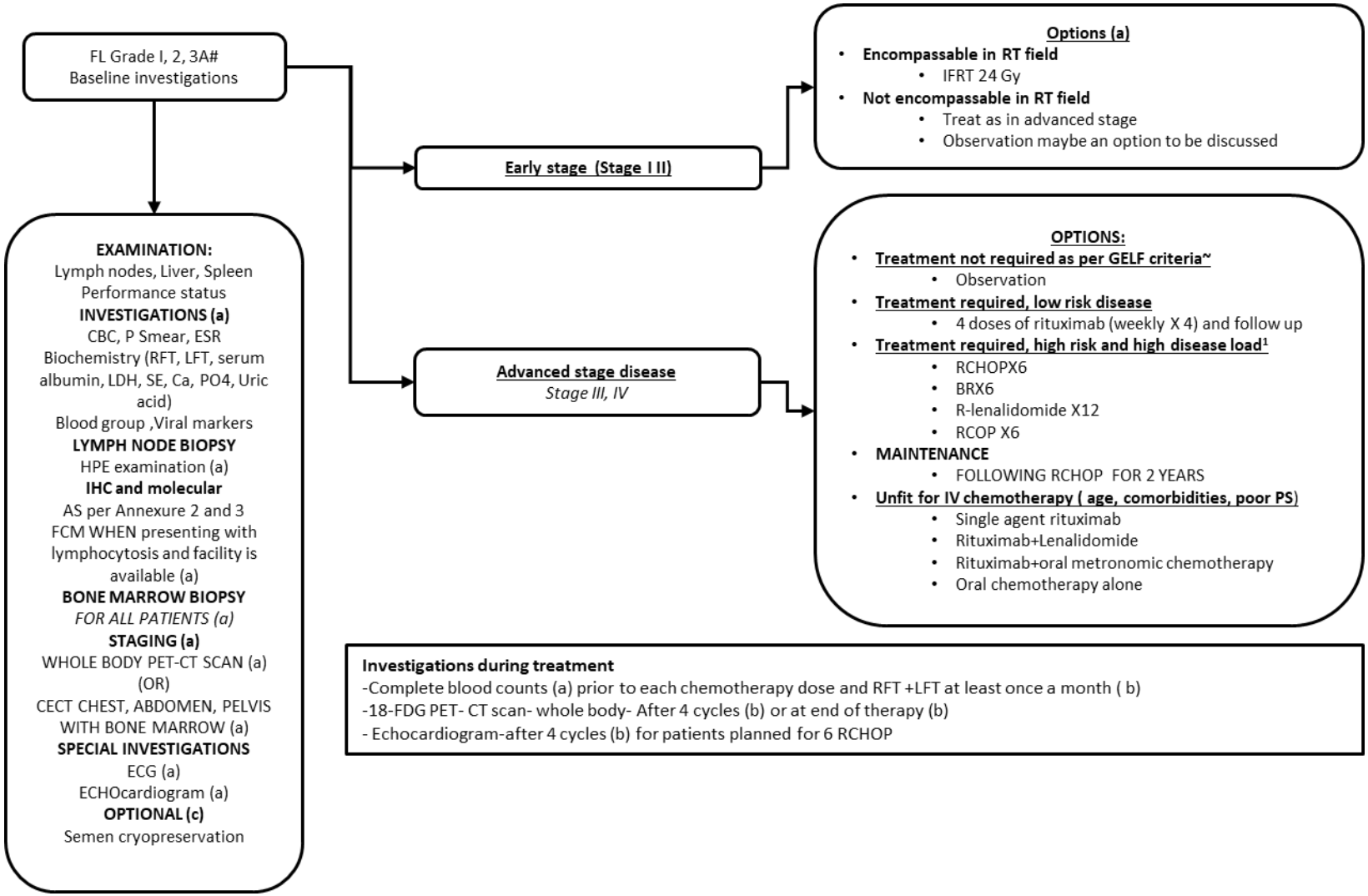
*CSF study at baseline for all patients with double hit lymphoma, bone marrow involvement, kidney, adrenal, testis, thyroid or breast involvement, HIV positive, CNS_IPI >3

1. To be discussed in MDT for tailoring of therapy. RT must be considered for bulky disease (>7 cm)
2. Requirements for HDMTx- see annexure 4.

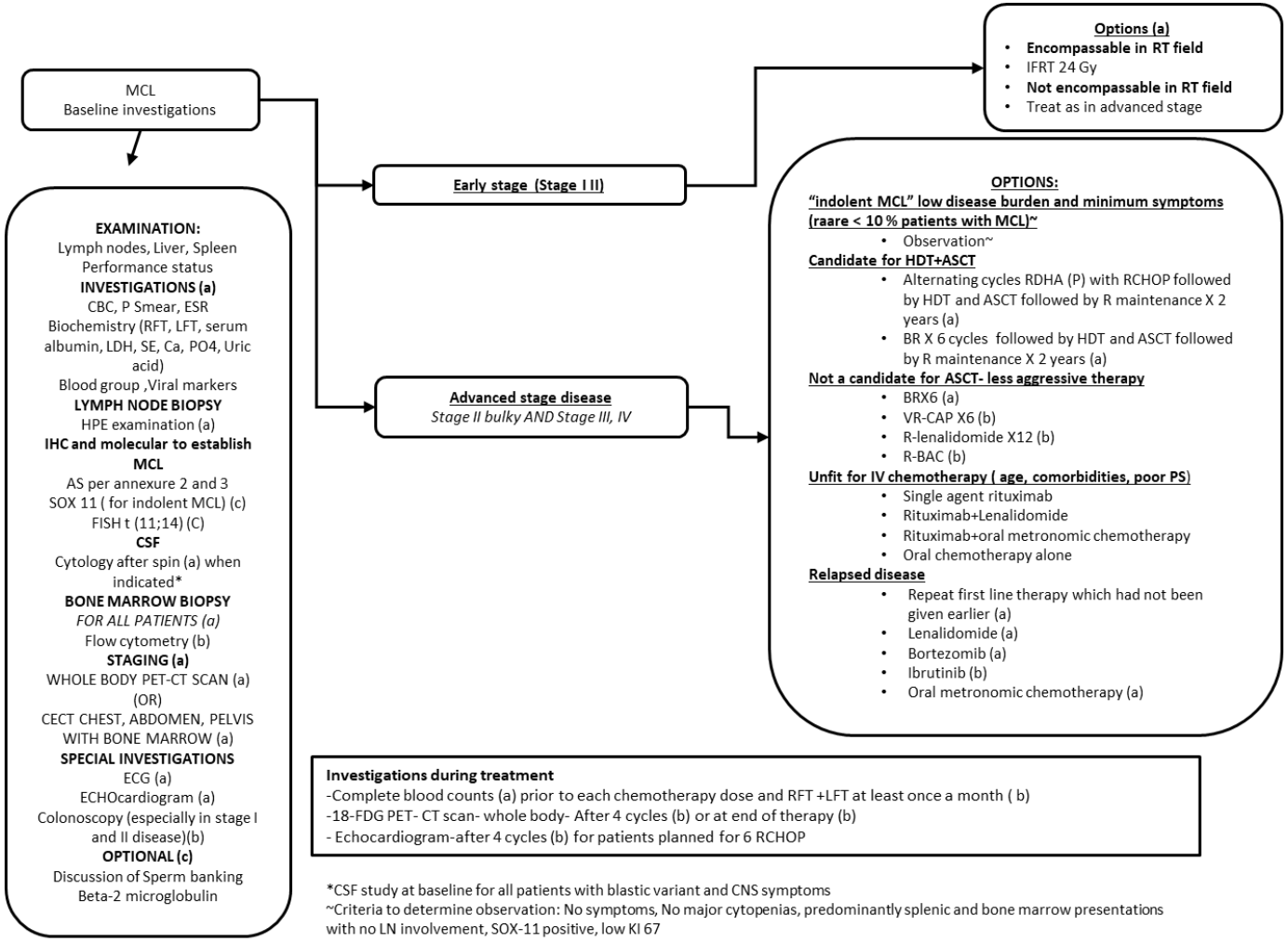
DLBCL-2- relapsed DLBCL



Follicular Lymphoma

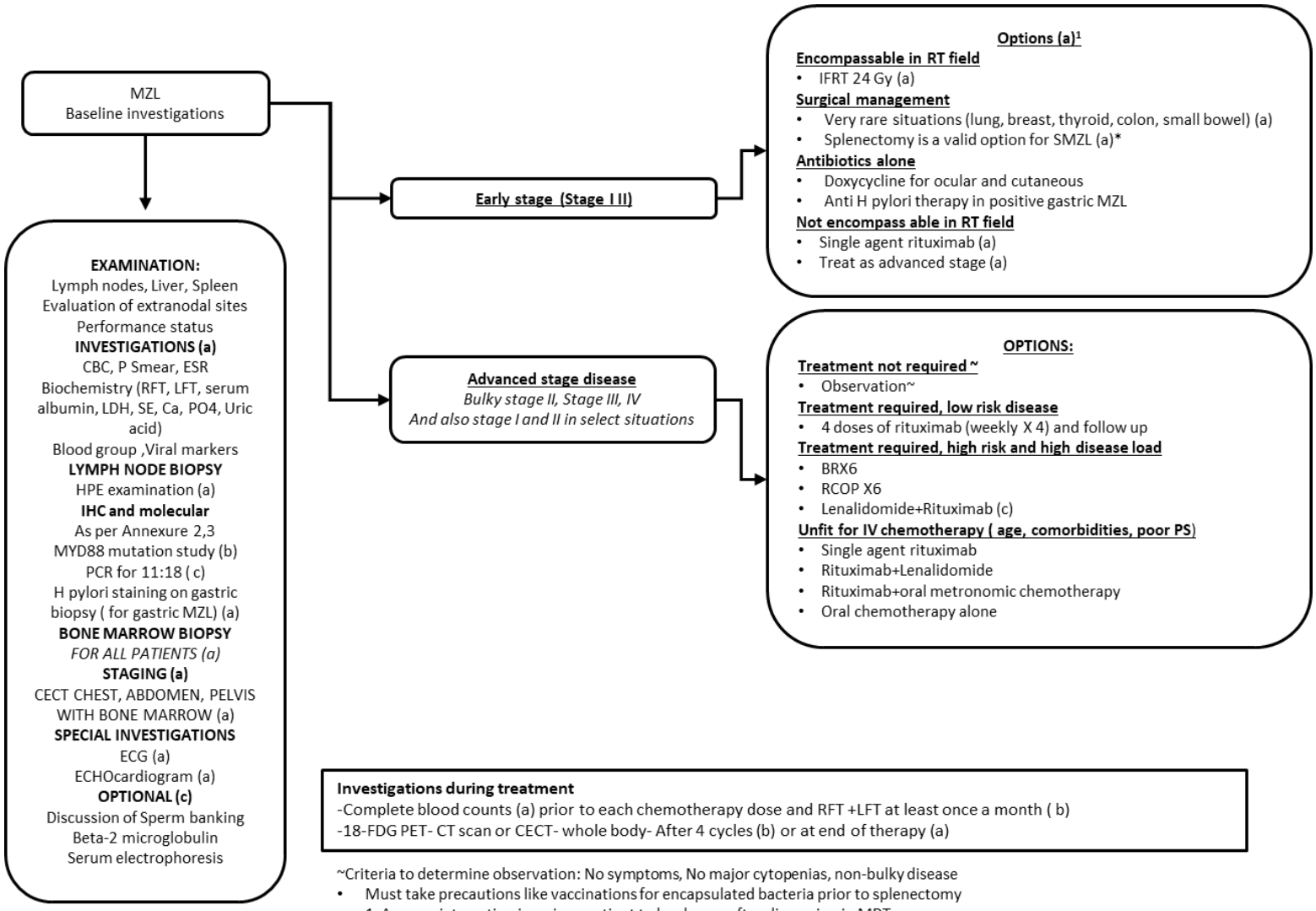


Mantle Cell Lymphoma



*CSF study at baseline for all patients with blastic variant and CNS symptoms
~Criteria to determine observation: No symptoms, No major cytopenias, predominantly splenic and bone marrow presentations with no LN involvement, SOX-11 positive, low KI 67

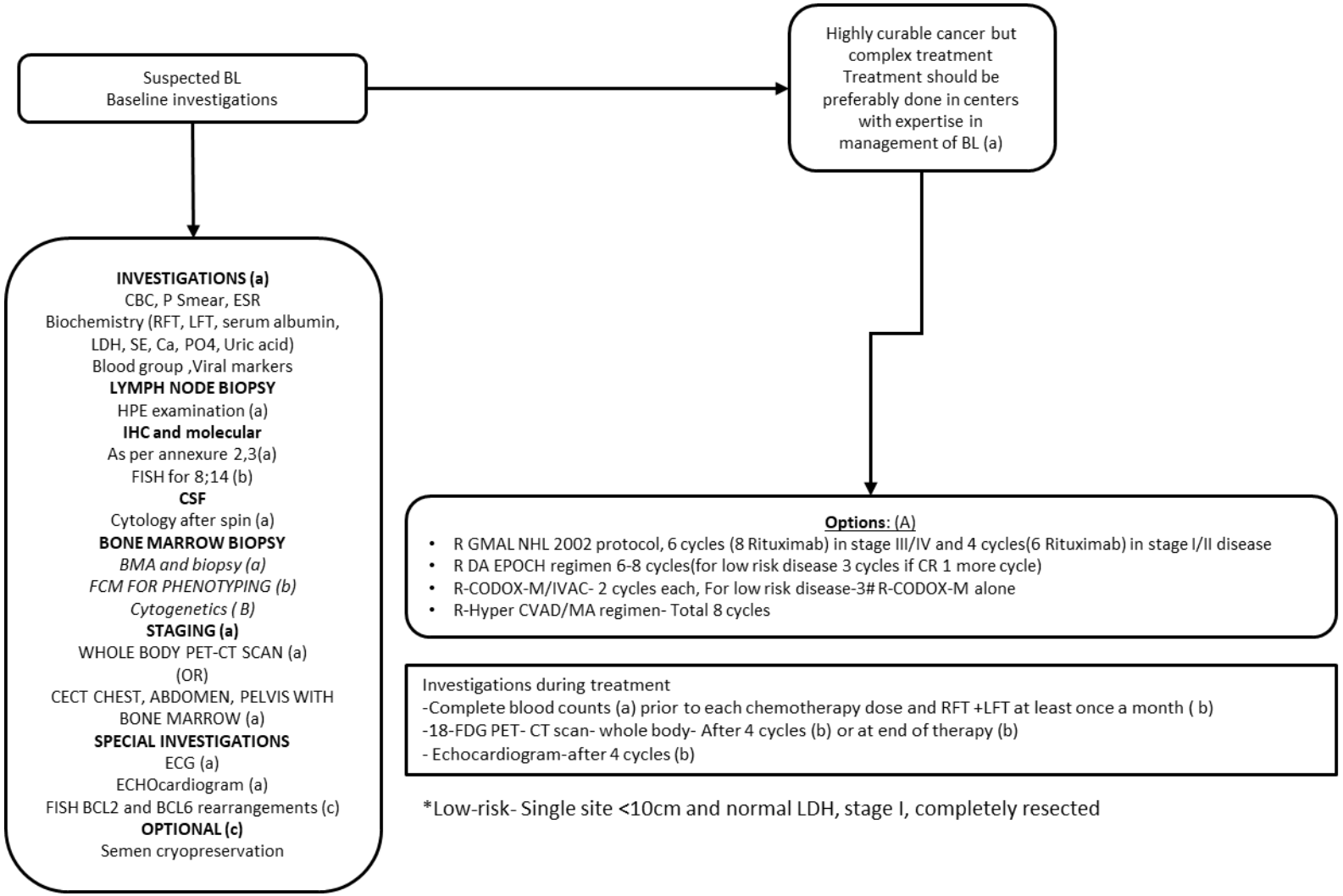
Marginal Zone Lymphoma



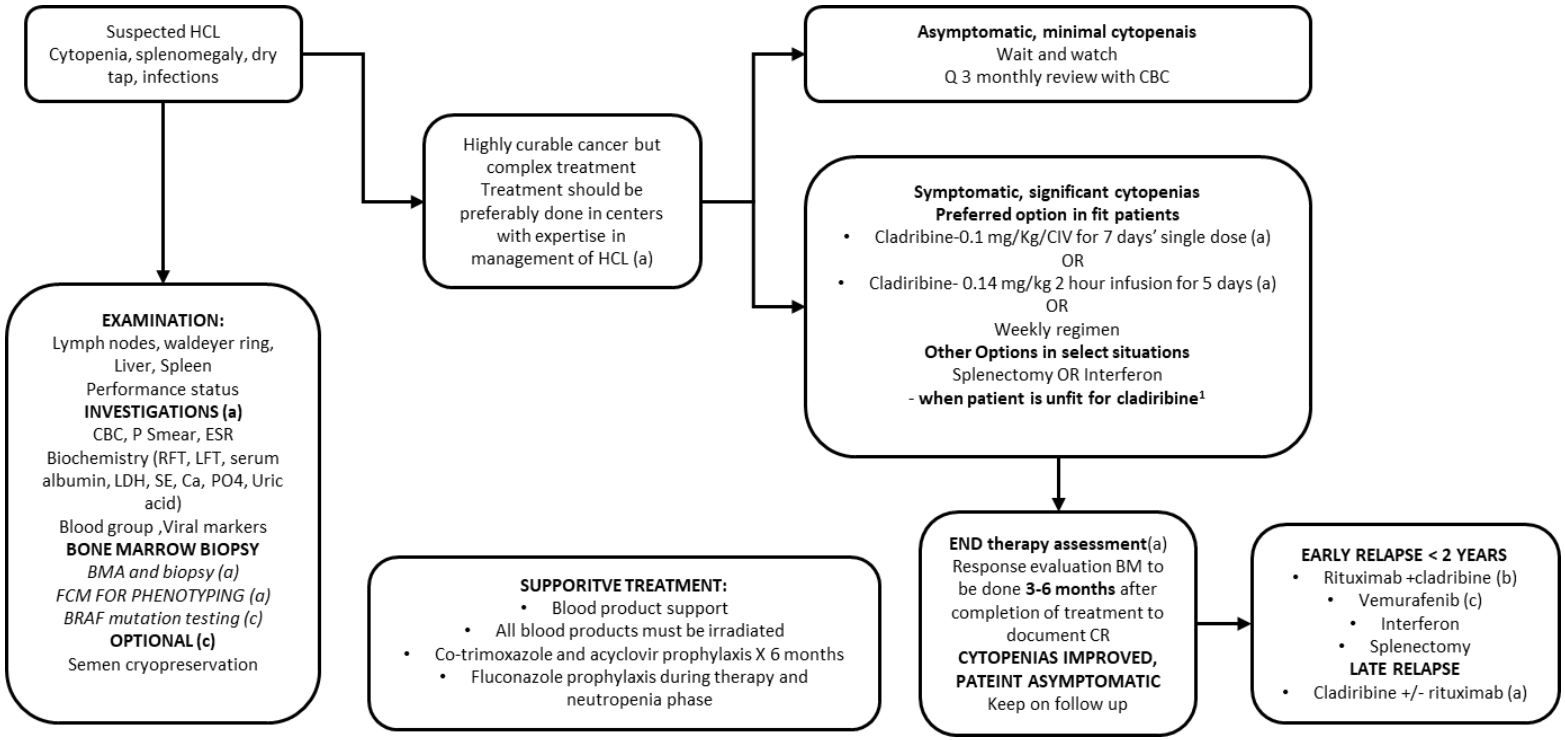
~Criteria to determine observation: No symptoms, No major cytopenias, non-bulky disease

- Must take precautions like vaccinations for encapsulated bacteria prior to splenectomy
- 1. Appropriate option in a given patient to be chosen after discussion in MDT

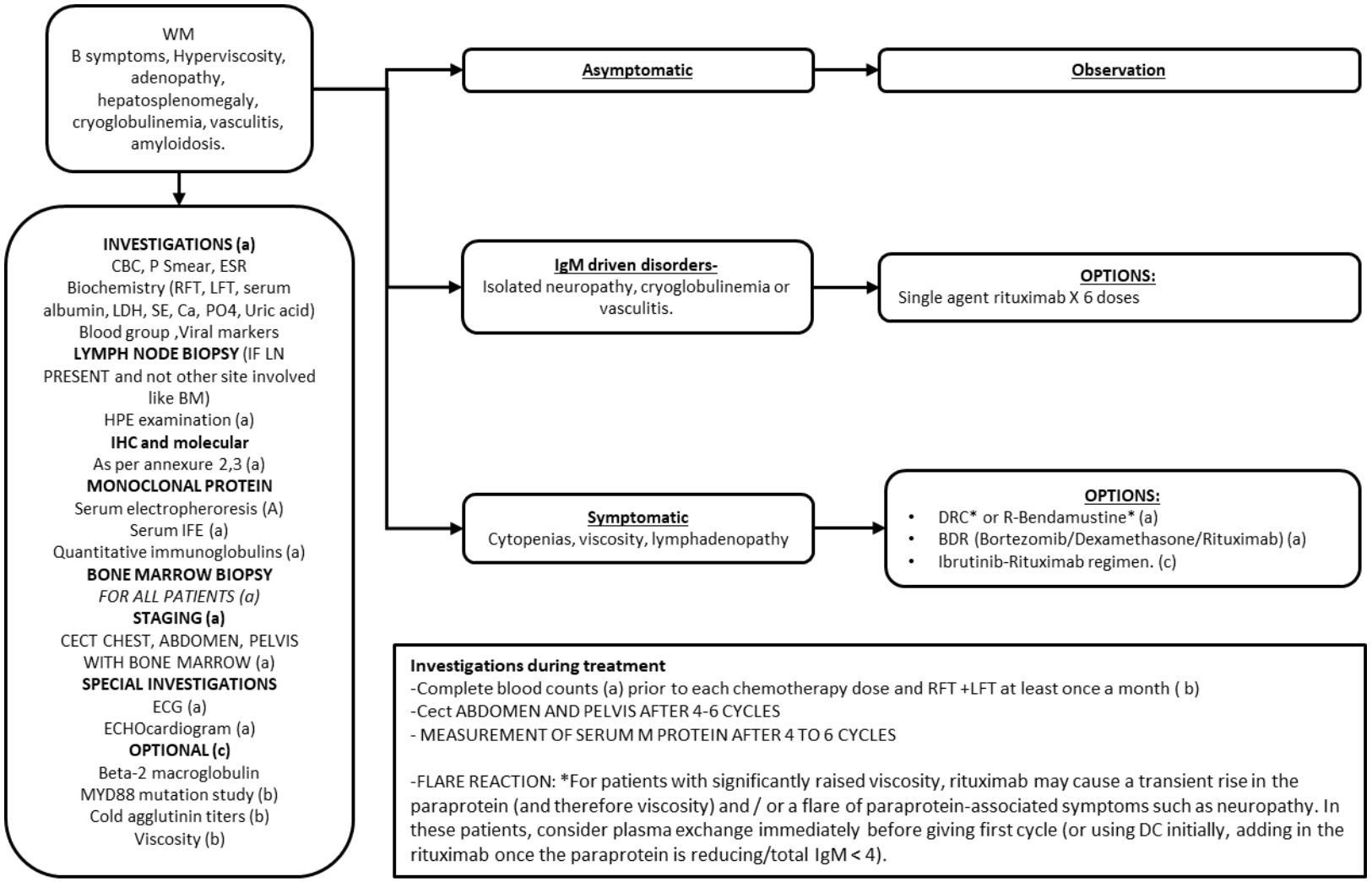
Burkitt's Lymphoma



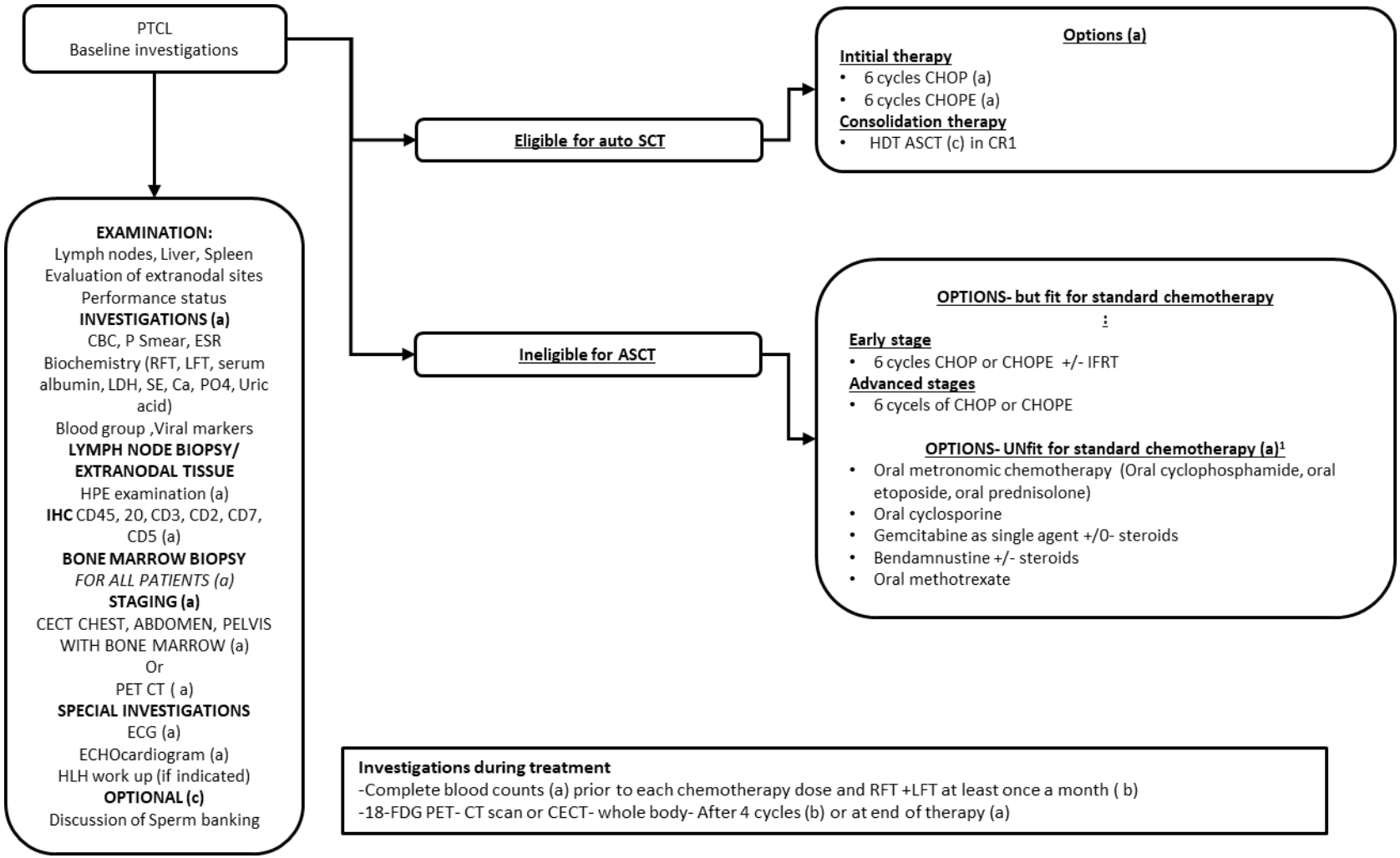
Hairy Cell Leukemia



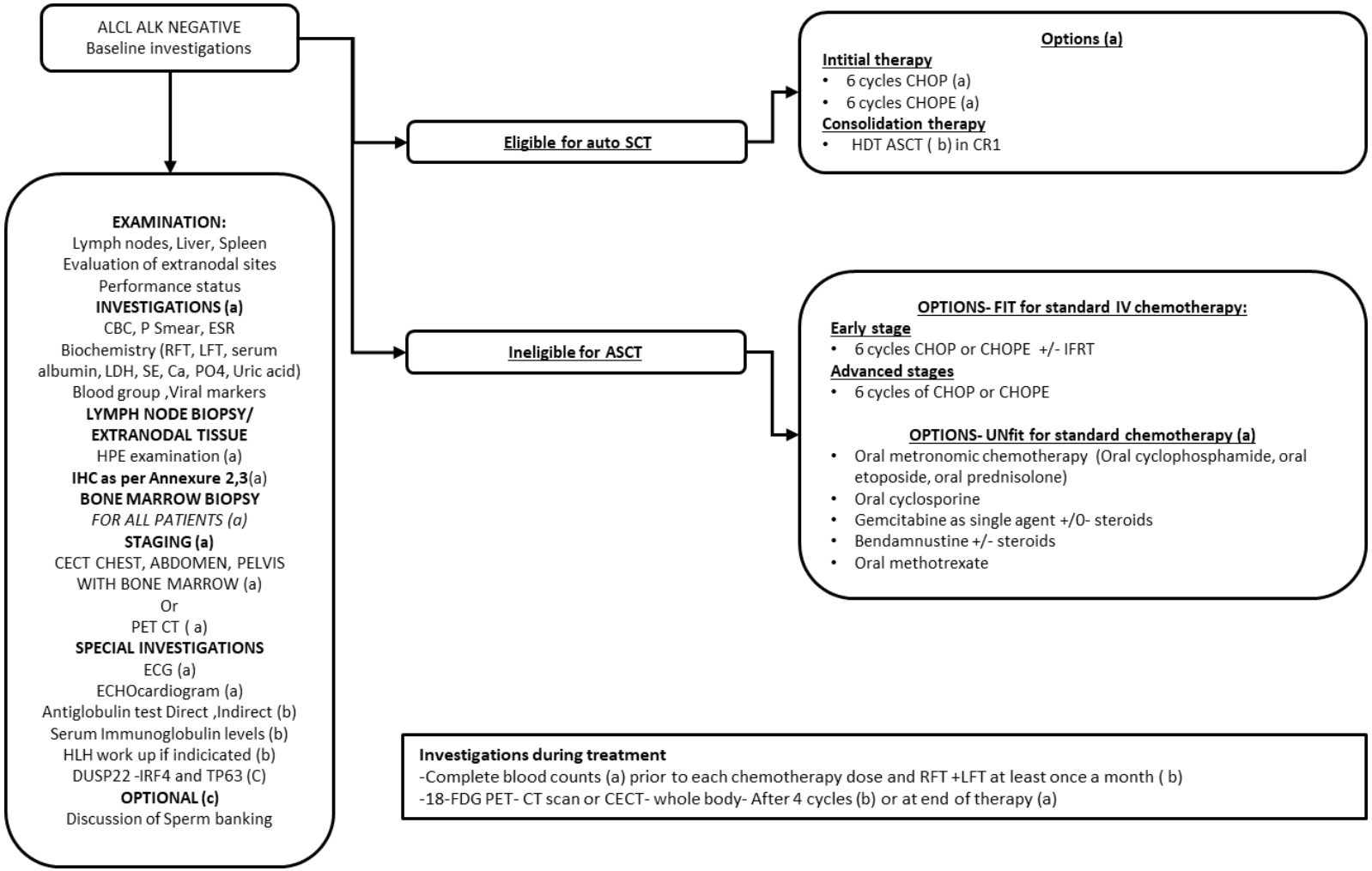
Waldenstroms Macroglobulinemia



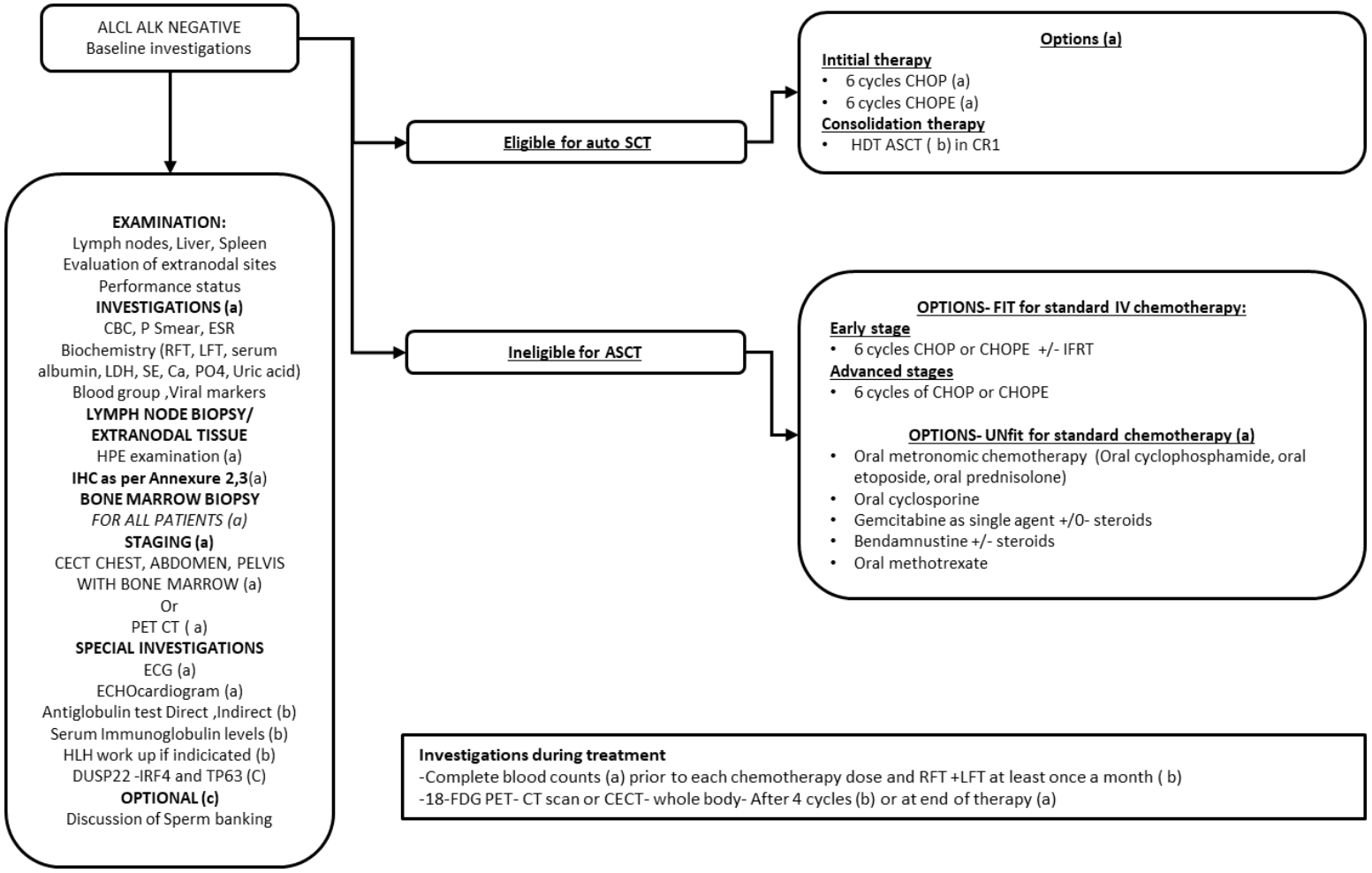
Peripheral T cell Lymphoma (NOS)

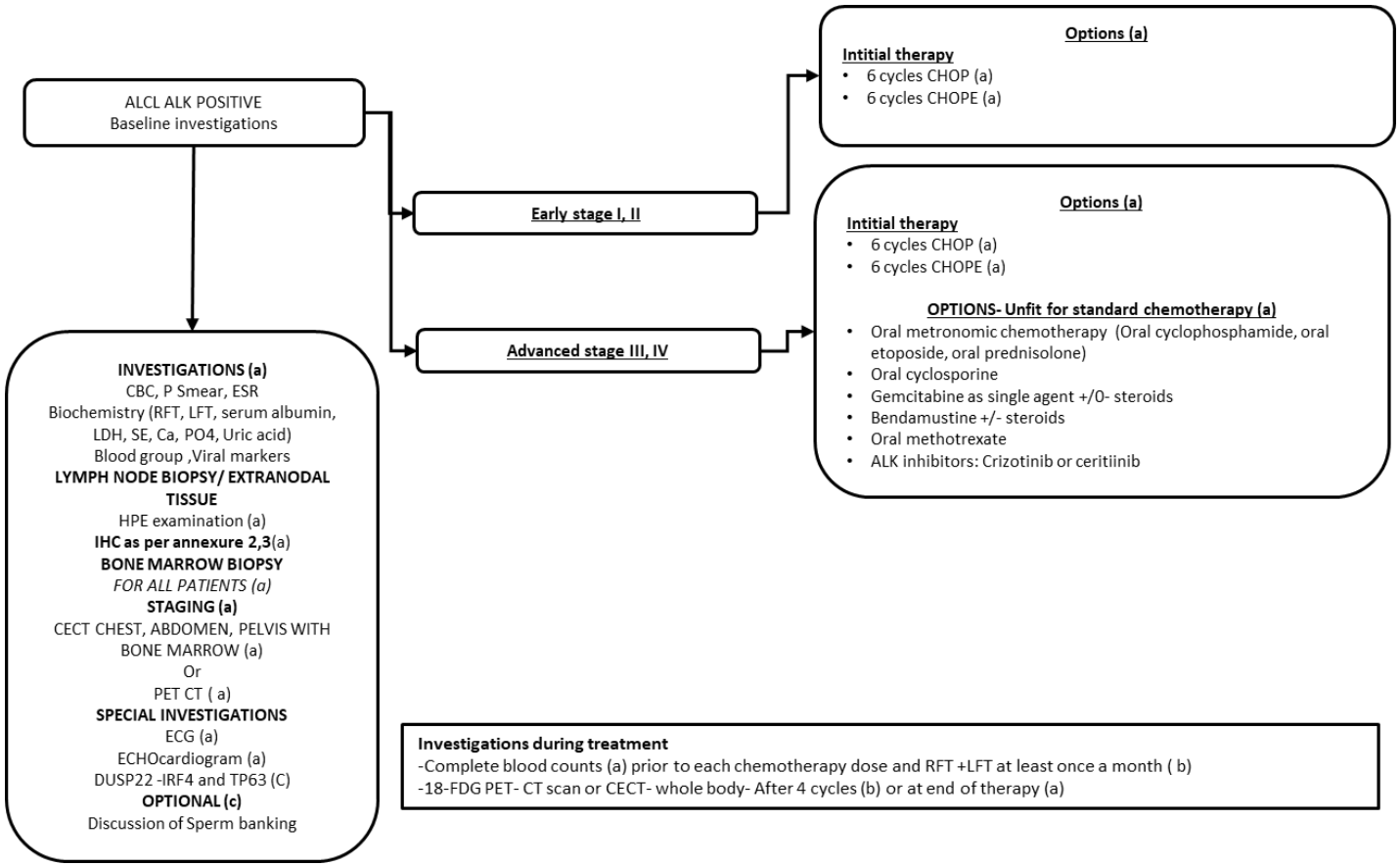


Angioimmunoblastic T cell Lymphoma



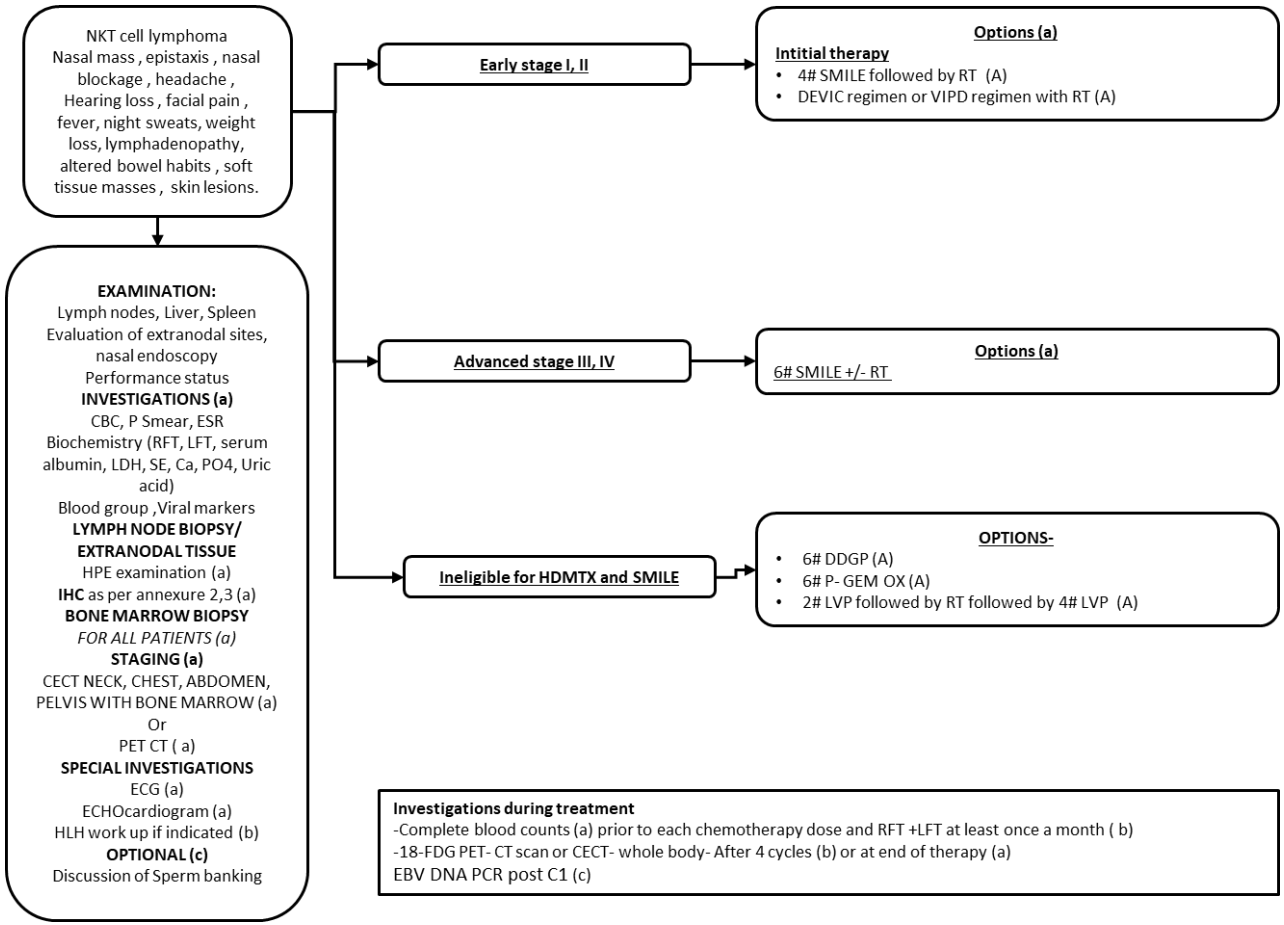
ALCL-1 Anaplastic Large Cell Lymphoma ALK negative





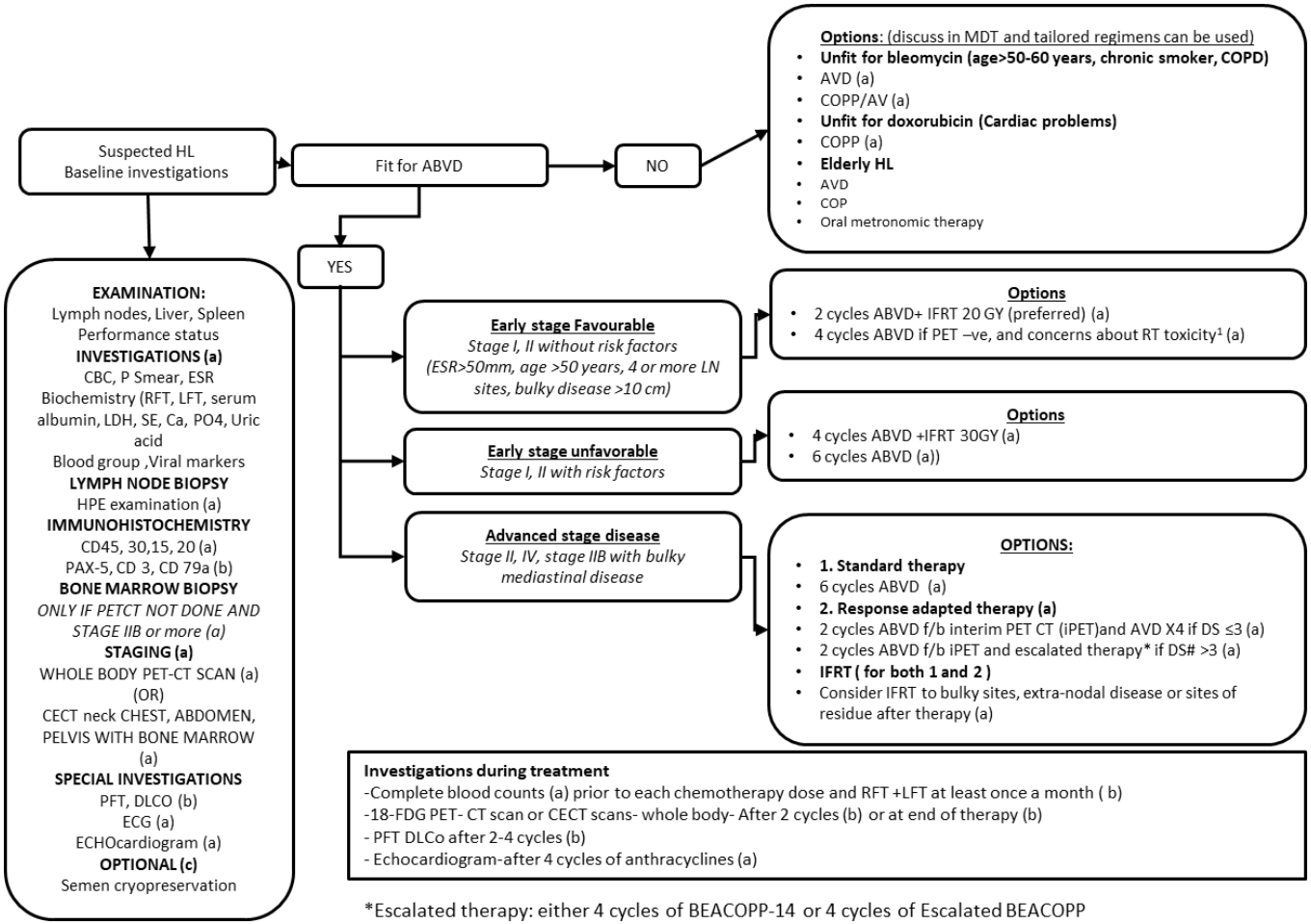
ALCL-2 Anaplastic Large Cell Lymphoma ALK positive

NK-T cell Lymphoma



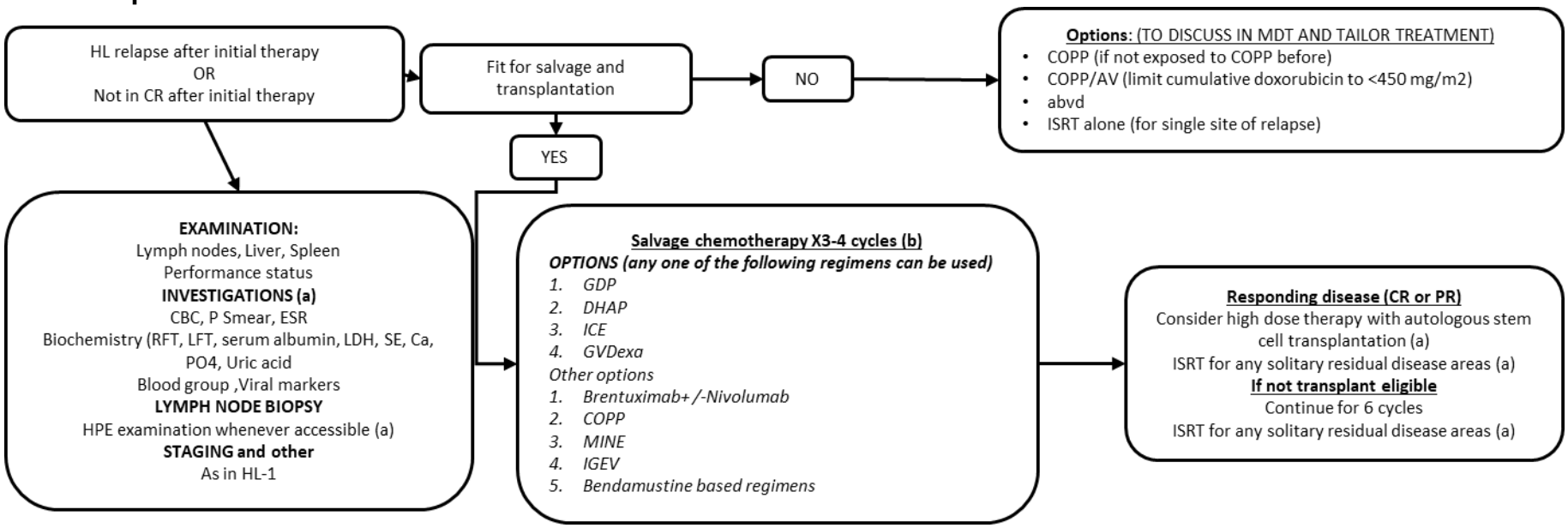
Hodgkin's Lymphoma

HL-1 Newly diagnosed HL



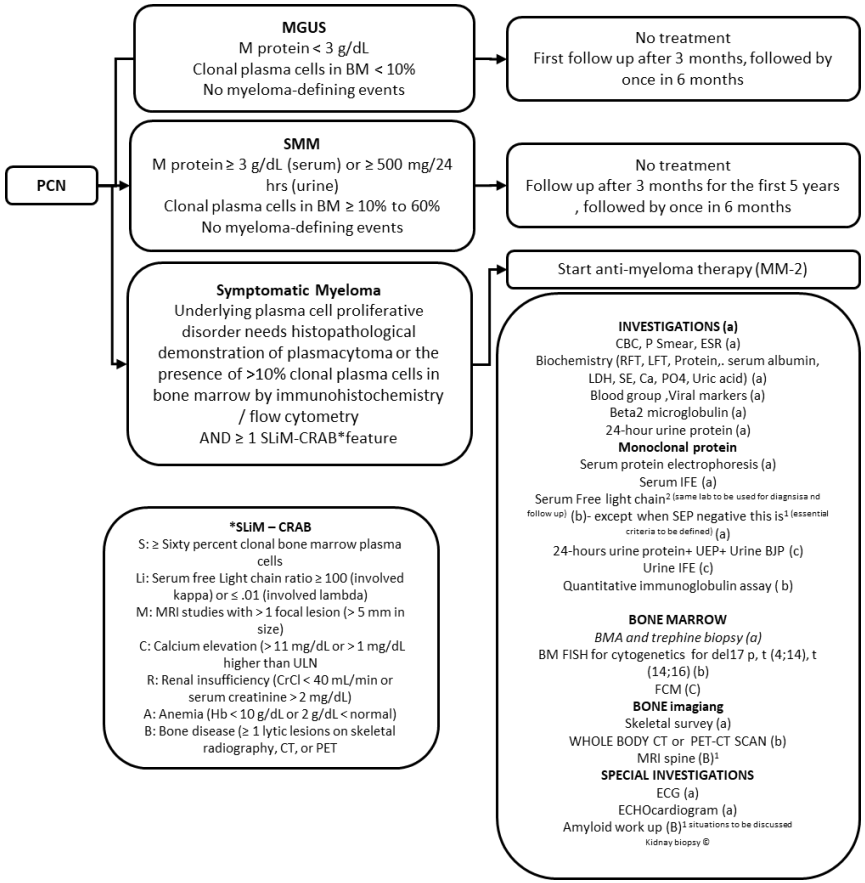
*Escalated therapy: either 4 cycles of BEACOPP-14 or 4 cycles of Escalated BEACOPP
#DS: Deauville score
1. Eg. Mediastinal mass in a female- concerns about RT exposure to breast

HI-2- Relapsed HL

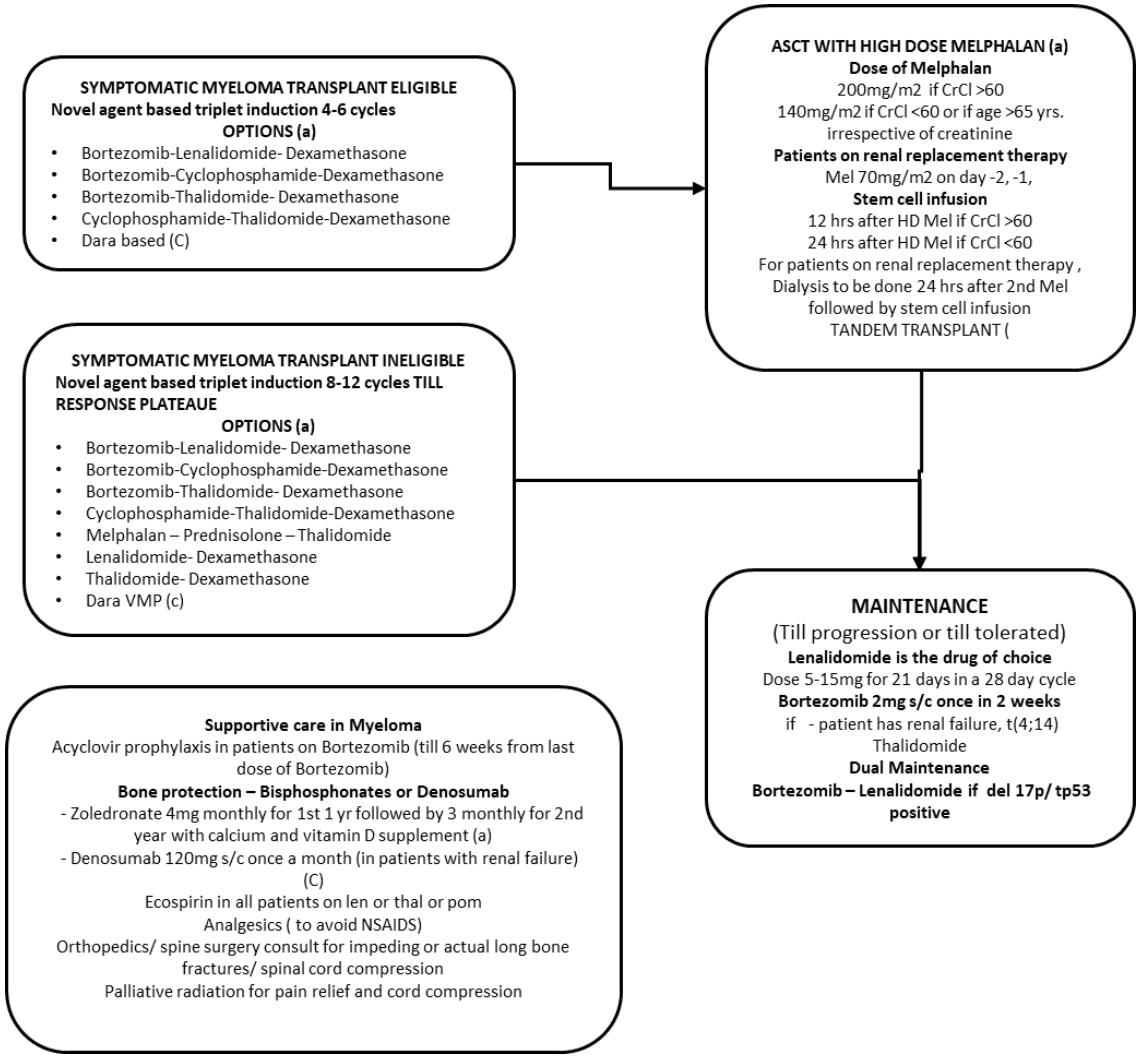


Plasma Cell Disorders

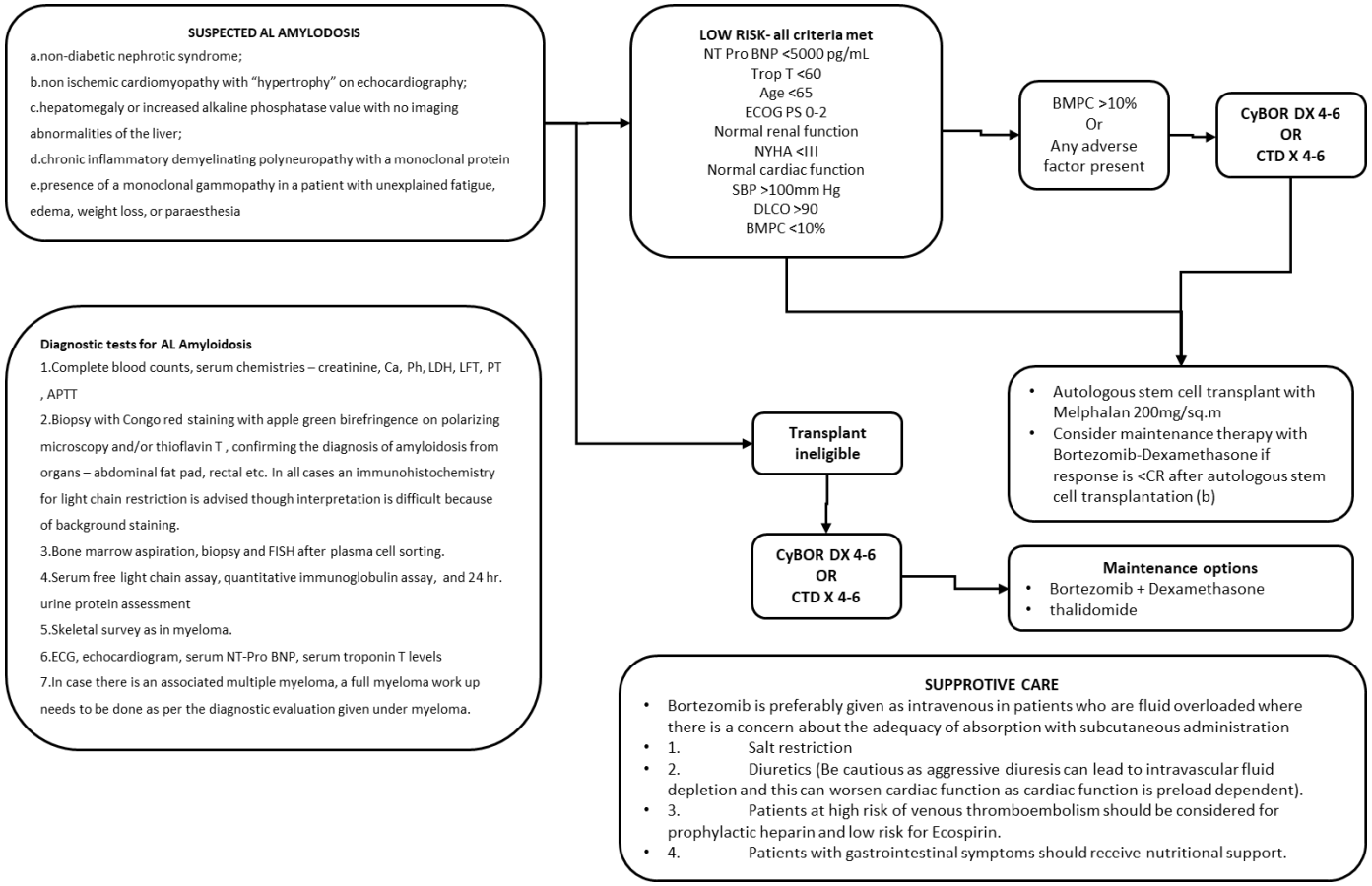
MM-1 MYELOMA, MGUS, SMOLDERING MYELOMA



MM-2- INITIAL THERAPY OF SYMPTOMATIC MYELOMA



AL AMYLOIDOSIS



Response assessment in primary systemic amyloidosis

Response	Definition
Haematological criteria	
Complete response	Negative serum and urine immunofixation and normal serum FLC ratio

Very good partial response	dFLC <40
Partial response	Reduction in FLC >50% compared with baseline
No response	All other scenario
Cardiac response	Decrease of NT-pro BNP by >30% and 300 ng/L (if baseline NT-pro BNP > 650 ng/L), or at least 2-point decrease of NYHA class (if baseline NYHA class is III or IV)
Renal response	At least 30% decrease in proteinuria or drop below 0.5 g/24 h, in the absence of renal progression defined as a >25% decrease in eGFR
Hepatic Response	50% decrease in alkaline phosphatase Reduction in liver size by 2cm radiographically

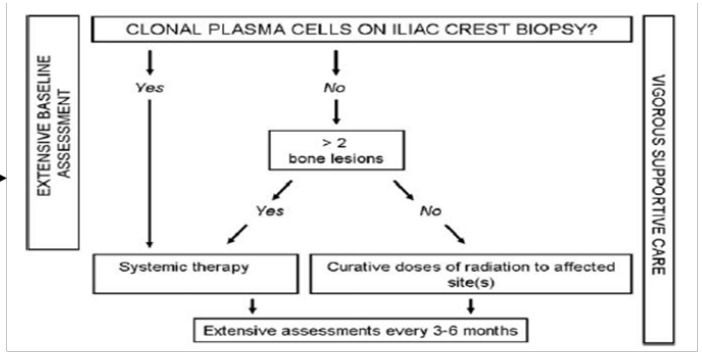
dFLC – Difference between involved and uninvolved free light chains.

POEM SYNDROME

POEM SYNDROME

FULFILS DIAGNOSTIC CRITERIA

A diagnosis of POEM syndrome is confirmed when **both of the mandatory criteria**, **one of the three major criteria**, **and one of the six minor criteria are present**.



Investigations at diagnosis

Complete blood counts
S. Creatinine
S. Calcium
Total protein/ Albumin
Serum protein electrophoresis
Serum Free light chain
Immunofixation electrophoresis
Bone marrow aspiration and trephine
Skeletal survey by x rays
Skull
Pelvis
Vertebrae
Long bones – Femur, Humerus, Clavicle
OR
Whole body low dose CT/ PET CT.

Optional regimens in POEMS

- Cyclophosphamide – Bortezomib- Dexamethasone
- Lenalidomide – Dexamethasone
- Bortezomib – Dexamethasone

Mandatory (Both needed)	1. Polyneuropathy (Typically demyelinating) – seen in all patients (peripheral, ascending, symmetrical, and affecting both sensation and motor function should be elicited) 2. Monoclonal plasma cell proliferative disorder(almost always λ)
Major (1/3 needed)	1. Castleman disease [a] 2. Sclerotic bone disease 3. Elevated VEGF levels
Minor (1/6 needed)	1. Organomegaly (Hepatomegaly, Splenomegaly, lymphadenopathy) 2. Extravascular fluid overload (edema, pleural effusion, ascites) 3. Endocrinopathy [b] (adrenal, thyroid, pituitary, gonadal, parathyroid, and pancreatic) 4. Skin changes (Hyperpigmentation, hypertrichosis, glomeruloid haemangiomata, plethora, acrocyanosis, flushing, and white nails) 5. Papilledema (seen in 1/3 of patients) 6. Thrombocytosis/polycythaemia
	a. There is a Castleman disease variant of POEMS syndrome that occurs without evidence of a clonal PCD that is not accounted for in this table. This entity should be considered separately. b. Because of the high prevalence of diabetes mellitus and thyroid abnormalities, this diagnosis alone is not sufficient to meet this minor criterion.

RESPONSE ASSESSMENT IN POEMS SYNDROME

Hematologic response:

●Complete response (CRH) – Negative bone marrow and negative immunofixation of the serum and urine. Patients are not required to have a repeat bone marrow aspirate if the baseline bone marrow was negative.

●Very good partial response (VGPRH) – A 90 percent reduction in the M-protein or immunofixation positive only as long as M-protein was at least 0.5 g/dL at baseline.

●Partial response (PRH) – A 50 percent reduction in M-protein or immunofixation positive as long as baseline M-protein was at least 1.0 g/dL.

●No response – Less than a PRH.

VEGF response:

●Complete response (CRV) – Normalization of VEGF (<87 pg/mL).

●Partial response (PRV) – Decrease of ≥50 percent (baseline must be ≥200 pg/mL).

●No response (NRV) – Less than a PRV.

Radiologic response by FDG-PET:

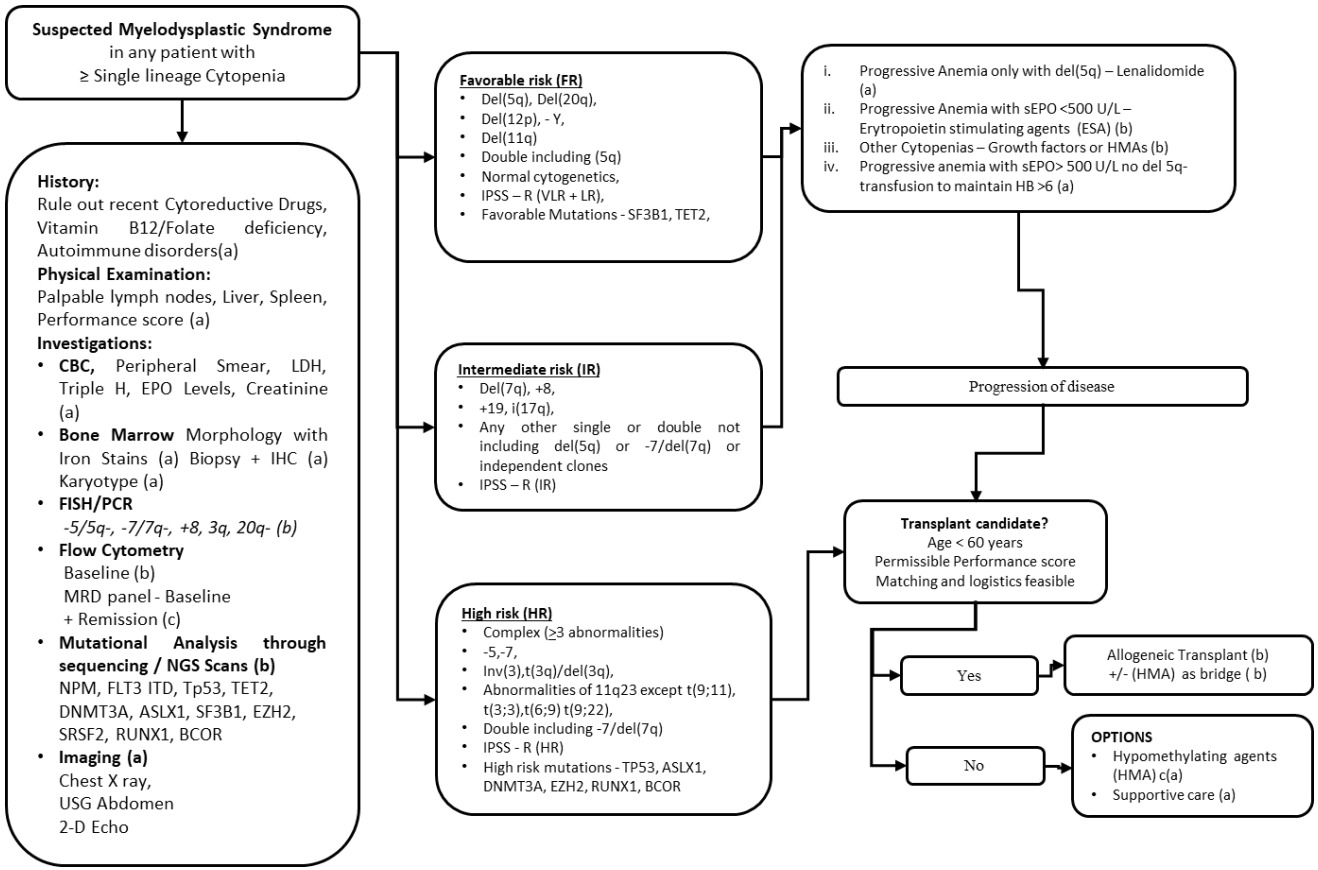
●Complete radiologic response (CRR) – Initial FDG avidity on a baseline PET scan that disappears.

●Partial radiologic response (PRR) – Initial FDG avidity that was 50 percent improved.

●No radiologic response – Not meeting CRR or PRR.

Clinical response: A clinical response assessment incorporates information regarding peripheral neuropathy, organomegaly, papilledema, erythrocytosis, thrombocytosis, endocrinopathy, extravascular fluid overload (ascites, effusions, edema), and abnormal pulmonary function tests. There are four clinical response categories, which include clinical improvement (IC), clinical progression (PC), mixed clinical response (MC), and clinical stability (SC).

MYELODYSPLASTIC SYNDROME



Annexure -1. Radiology Synoptic reporting formats

Myeloma MRI
Template for reporting of Multiple Myeloma.

WHOLE BODY MRI EXAMINATION.

Indication: work up for multiple myeloma / response assessment

Technique: Myeloma protocol – whole spine T1 T2 and STIR, whole body axial or coronal T1 gradient Dixon (5mm), whole body axial diffusion, whole body axial T2 5mm (optional), additional sequences for regional assessment (optional)

Comparison:

Findings:

Evaluation of bones: Spine and then head to thigh in descending order.

Measurement of up to 5 focal lesions and pattern of marrow infiltration: Normal / focal/ focal on diffuse/ diffuse/ micronodular

Paramedullary or extramedullary sites: Site with measurement

Vertebral fractures: Document presence and benign / malignant

Response assessment Categories (RAC) for each anatomic region: Cervical / thoracic / lumbar spine, pelvis, long bones, skull, ribs.

Posterior iliac crest: Is trephine likely to be representative?

Incidental findings:

Conclusion:

Summary statement, RAC score according to anatomical regions, heterogeneity, recommendations including for investigation of equivocal findings.

State level of concern regarding incidental findings.

MY-RADS response assessment categories

Response assessment category (RAC) description:

1: Highly likely to be responding

Return of normal fat containing marrow in areas previously infiltrated by focal or diffuse myelomatous infiltration

Unequivocal decrease in number or size of focal lesions

Conversion of a packed bone marrow infiltrate into discrete nodules, with unequivocal decrease in tumour load in the respective bone marrow space

Decreasing soft tissue associated with bone disease

Emergence of intra-or peritumoral fat within/ around focal lesions (fat.dot or halo signs)

Previously evident lesion shows increase ADC in from <= 1400 microm square /sec to >1400 microm square /sec

>= 40% increase in ADC from baseline with corresponding decrease in normalized high b value signal intensity; morphologic findings consistent with stable or corresponding disease

For soft tissue disease, RECIST version 1.1 criteria for PR/CORONA RADIATA

2: Likely to be responding

Evidence of improvement but not enough to fulfil criteria for RAC 1. For example:

Slight decrease in number / size of focal lesions

Previously evident lesions showing increase in ADC from <= 1000 microm square /sec to <1400 microm square /sec

>25% but <40% increase in ADC from baseline with corresponding decrease in high b value signal intensity:

Morphologic findings consistent with stable or responding disease

For soft tissue disease, RECIST version 1.1 not meeting requirement for PR

3: Stable

No observable change

4: Likely to be progressing

Evidence of worsening disease, but not enough to fulfil criteria for RAC 5

Equivocal appearance of new lesion (s)

No change in size but increasing signal intensity on high b value (with ADC values <1400 microm square /sec) consistent with possible disease progression

Relapsed disease : reemergence of lesion (s) that previously disappeared or enlargement of lesion (s) that had partially regressed / stabilized with prior treatments.

Soft tissue in the spinal canal causing narrowing noted associated with neurological findings and not requiring radiation therapy

For soft tissue disease, RECIST version 1.1 not meeting requirement for PD

5: Highly likely to be progressing

New critical fracture(s) / cord compression requiring radiation / surgical intervention ; only if confirmed as malignant with MRI signal characteristics

Unequivocal new focal (> 5 to 10mm) / diffuse area (s) of infiltration to regions of previously normal marrow

Unequivocal increase in number/ size of focal lesions

Evaluation of focal lesion to diffuse neoplastic pattern

Appearance / increasing soft tissue associated with bone disease

New lesions / region of high signal intensity on high b-value images with ADC value between 600-1000 microm square /sec

Reference:

Christina Messiou, Jens Hillengass, Stefan Delorme, et al. Guidelines for acquisition, interpretation, and reporting of whole-body MRI in myeloma: Myeloma response assessment and diagnosis system (MY-RADS) Radiology 2019;291(1):5-13.

Myeloma Whole body CT

Whole body low dose CT for multiple myeloma assessment.

Technique:

A few details about the technique used should be given, including number of detectors, slice thickness, anatomy scanned (e.g., skull to proximal tibial metaphyses), and whether MPRs were performed. If prominent artifacts degrade significantly the image quality in certain parts of the anatomy, this should be specifically stated- eg

Recommended technical parameters.

Whole body low dose plain CT should be performed from the cranial vault to at least proximal metaphysis of the tibia on a multidetector scanner with 16 detector rows or more, using kv 120 and 50 to 70 mAs. The collimation must be set between 0.5 and 1.5 mm and images are reconstructed in bone and soft tissue algorithm. Sagittal MPRs of the bone algorithm images of the whole spine and additional MPRs reconstructed parallel to the long axis of the femora and humeri are performed.

Table for technical parameters:

Number of detector rows	16 or more
Scan coverage	Cranial vault to proximal tibial metaphysis (include humeri in the field of view)
Tube voltage(kV)/time-current product (mAs)	120/50–70a
Collimation	0.5–1.5 mm
Reconstruction convolution kernel	Sharp, high-frequency (bone) and smooth (soft tissue). Alternatively, one middle-frequency kernel for all images
Iterative reconstruction algorithms	Yes (to reduce image noise and streak artifacts)
Thickness/increment of axial slices	2/1 mm or 3/1.5 mm

Multiplanar Reconstructions (MPRs)	Yes (sagittal, coronal and parallel to long axis of proximal limbs).

Different tube parameters (e.g., 140/14–25 or a low voltage approach) are acceptable as long as they produce images of diagnostic quality with low effective patient dose

Indication : work up

Clinical information/Prior imaging studies

Findings:

Skull:

Spine: Cervical –

Thoracic-

Lumbar-

Sacral-

Upper limbs-

Ribs-

Stermun:

Scapula:

Pelvic bones-:

Lower limbs:

Visceral assessment:

Extramedullary disease:

Other findings:

Bones should be commented upon for-

- Osteolytic lesion - presence / absence, size of main lesions

-Focal and/or diffuse intramedullary hyperdensities of the femora and humeri - present / absent, if present the location, size, density and presence/absence of significant endosteal scalloping

-Extraosseous soft tissue with spinal compromise if any

-Increased fracture risk due to the presence of extensive osteolysis, especially in weight-bearing bones such as those of the lower spine and lower limbs should be mentioned.

-Fractures and associated complications

Conclusion

A clear summary statement should highlight the most important findings regarding overall disease status. It should include number and distribution of osteolyses, presence/absence of extraosseous soft tissue masses, likelihood of cord/nerve root compression, number of focal medullary deposits in the appendicular skeleton, presence/absence of diffuse medullary disease in the appendicular skeleton and a comment on vertebral compression fractures and/or vertebral fracture risk.

Appropriate recommendations.

Reference:

Recommendations for acquisition, interpretation and reporting of whole body low dose CT in patients with multiple myeloma and other plasma cell disorders: a report of the IMWG Bone Working Group

LA Moulopoulos, Vassilis Koutoulidis et al.

Blood Cancer Journal **volume 8**, Article number: 95 (2018)

Myeloma – skeletal survey

Skeletal Survey

Frontal and lateral views of the skull, cervical, thoracic and lumbar spine, bilateral oblique views of the ribs, and frontal views of the upper and lower extremities.

Clinical information

Comparison

Findings

Skull:

Cervical spine:

Thoracic spine:

Lumbar spine:

Bilateral oblique views of the ribs:

Right upper extremity:

Left upper extremity:

Right lower extremity:

Left lower extremity:

Bones are described in relation to :

Lytic lesions – present or absent (if present location)

Fractures / Bone density / associated soft tissue

Impression

Communication of findings:

Lymphoma- CT scan

CT template for lymphoma assessment:

CT SCAN OF NECK, CHEST, ABDOMEN AND PELVIS

Post contrast CT scan of neck, chest abdomen and pelvis has been performed from skull base to ischial tuberosity.

Indication: Staging / response assessment of lymphoma.

Comparison:

Findings:

Neck

Nodes: present / absent

If present – laterality / level/ longest dimension of the largest node

Pharynx and larynx:

Oral cavity and tonsils:

Salivary glands:

Thyroid:

Vessels and carotid space:

Thorax

Lungs:

Mediastinal and hilar nodes: Absent / present

If present: location / size of nodal mass in maximum dimension or transverse diameter of nodal mass excluding the normal structures/ extension to adjacent structures

Trachea and bronchi:

Pleural spaces:

Heart and pericardium:

Vessels: Thrombus present / absent

Oesophagus:

Chest wall:

Axillary nodes:

<u>Abdomen and Pelvis</u>

Nodes: retroperitoneal / mesenteric/ iliac / inguinal nodes - Site/ size of largest node or nodal mass in maximum dimension

Liver: normal / enlarged in size.

attenuation- normal / fatty

focal lesion - present / absent

vessels- normal / periportal infiltration

Spleen: normal/ enlarged ; if enlarged size

focal lesion- present/ absent

Gall bladder:

Adrenals:

Pancreas:

Kidneys and ureters: normal / enlarged ; hydronephrosis- present/ absent

focal lesion: present / absent

perirenal space: normal / soft tissue infiltration

Stomach and bowel: unremarkable/ wall thickening / aneurysmal dilatation

Peritoneum and omentum:

Urinary bladder:

Pelvic organs:

Ascites:

Bones: normal / lytic or sclerotic lesion

<u>Conclusion:</u>

Staging if primary / Response assessment

Cotswolds modified Ann Arbor Staging Classification for both Hodgkin and non-Hodgkin lymphoma

CT response assessment should be based on RECIL 2017 criteria.

Cotswold's modified Ann Arbor Staging Classification for both Hodgkin and non-Hodgkin lymphoma

- **stage I:** one nodal group or lymphoid organ (e.g. spleen or thymus)
  - **stage IE:** one extranodal site
- **stage II:** two or more nodal groups, same side of the diaphragm
  - **stage IIE:** localized extranodal site with stage II criteria, both on the same side of the diaphragm
- **stage III:** nodal groups on both sides of the diaphragm
  - **stage IIIS(1):** with splenic involvement
  - **stage IIIE(2):** with localized extranodal site
  - **stage IIISE:** both
- **stage IV:** disseminated involvement of one or more extra lymphatic organ (e.g. lung, bone) with or without any nodal involvement

Additional sub-staging variables:

- **A:** asymptomatic
- **B:** presence of B symptoms (including fever, night sweats and weight loss of over 10% of body weight over 6 months)
- **X:** bulky nodal disease: nodal mass >1/3 of intrathoracic diameter or 10 cm in dimension

RECIL criteria for response assessment

Complete Response

- Complete disappearance of all target lesions and all nodes with a long axis < 10 mm
- ≥ 30% decrease in sum of longest diameters of target lesions (partial response) plus normalization of FDG-PET
- Normalization of FDG-PET (Deauville score 1–3)
- No bone marrow involvement
- No new lesions
- Reduction in the sum of diameters by ≤ 30% with normalization of FDG-PET uptake should not be considered a complete response unless documented by negative tissue biopsy.

Partial Response

- ≥ 30% decrease in the sum of longest diameters of target lesions but not a complete response
- Positive FDG-PET (Deauville score 4–5)
- Any bone marrow involvement
- No new lesions

Minor Response

- ≥ 10% decrease in the sum of longest diameters of target lesions but not a partial response
- Any FDG-PET findings
- Any bone marrow involvement
- No new lesions

Stable Disease

- < 10% decrease or ≤ 20% decrease in the sum of longest diameters of target lesions
- Any FDG-PET findings
- Any bone marrow involvement
- No new lesions

Progressive Disease

- > 20% increase in the sum of longest diameter of target lesions
- For small lymph nodes of < 15 mm post therapy, minimum absolute increase of 5 mm and long diameter > 15 mm
- Appearance of new lesion
- Any FDG-PET finding
- Any bone marrow involvement
- New or no new lesions

Infection assessment- HRCT chest

HRCT/ PLAIN CT SCAN OF THORAX

HRCT / Plain CT scan of thorax has been performed.

Indication: To look for infective focus.

Comparison:

Findings:

Lungs:

Consolidation- Absent/ Lobar/ segmental / sub segmental

Nodules- Absent / Discrete/ tree in bud / nodules with surrounding ground glass

Patchy ground glass density- Present /Absent: if present distribution.

Septal thickening- Present / Absent, distribution

Pleura: effusion / thickening

Heart and great vessels:

Mediastinal nodes:

Chest wall:

Visualized abdomen:

Visualized Bones:

Impression:

Chest findings if infective, if imaging is suggestive of possible etiology like bacterial or fungal

Any recommendations

Annexure 2. Lymphoma diagnostic and IHC panels

1. Lymphoma – Pre analytical requisites

Mandatory

- Tissue preservation (avoid frozen processing)
    - Fixative: 10% neutral buffered formalin
    - Fixation:
        - Lymph nodes/tissue thicker than 0.8 -1.0cms; should be bisected and large tissue should be serially sliced, perpendicular to the long axis.
        - Tissue ≤4 cm in greatest dimension should be processed in entirety
        - Should be put for fixation within 30 – 60 minutes of biopsy
        - Fixation volume should be at 3-4 times the volume of the tissue
        - Should not be left in the fixative for more than 48 hrs; and should be processed in 12-24 hrs time (in cases of inevitable delay; should be kept in cold temperature [refrigerator], preferably at 4 degrees centigrade)
- Routine processing and embedding
    - 3-5 micron thick sections with Hematoxylin and eosin stained slides of each paraffin block
    - Basic immunohistochemistry set up
    - Microscopic evaluation

Optional (Immunohistochemistry and Molecular diagnostic laboratories)

**For transportation – Either by immersing tissue in the adequate formalin in a sealed container or by paraffin blocks*

2. Lymphoma Diagnosis

Mandatory:

- Diagnosis:
    - Histological evaluation, i.e. biopsy as a method of investigation with comprehensive IHC panels.
    - Only in instances of inability of get adequate, a fine needle aspiration (FNA) based flow cytometric evaluation should be considered for diagnosis
- Staging
    - Bone marrow biopsy, aspirate and imprint smear

Optional/extended work-up:

- Diagnosis:
    - Fine needle aspiration (FNA) based flow cytometric immunophenotyping along with the biopsy
    - Molecular work-up
- Staging:
    - Flow cytometric immunophenotypic evaluation

3. Hodgkin lymphoma- cHL and NLPHL- requisites for diagnosis

Classic Hodgkin lymphoma (CHL)

- Mandatory:
    - CD3, CD20, CD30, CD15, Pax5, AE1/AE3*, ALK-1**
- Optimal/extended work-up:
    - LCA, CD3, CD20, CD30, CD15, Pax-5, Oct2, Bob1, EBV-LMP1/EBER, Gata 3

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)

- Mandatory:
    - CD20, CD3, CD30, CD15, Pax5
- Optimal/extended work-up:
    - CD3, CD20, CD30, CD15, Pax-5, EBVLMP1/EBER, PD1, Oct2, Bob1, Gata3, CD4, CD8

* Where ever indicated, to rule out a possibility of EBV related benign proliferations, poorly differentiated / undifferentiated carcinoma

** In cases of younger patients, rule out a possibility of ALK+ve ALCL

4. B cell NHL- requisites for diagnosis

A. CD20 positive BNHL: large cell morphology

- Mandatory:
    - IHC: LCA, CD20, CD3, MIB-1
- Optimal/extended work-up:
    - IHC: CD3, CD20, MIB-1, cyclin D1, CD5, CD10, Bcl6, Mum1, cmyc, Bcl-2, CD30, EBV-LMP1/EBER
    - FISH: CMYC/BCL2/BCL6 gene rearrangement
    - Gene expression/methylation studies – COO subtyping

B. CD20 positive BNHL: non-large cell morphology

- Mandatory:
    - IHC: LCA, CD20, CD3, MIB-1, CD5, CD23, CD10, bcl6, cyclin D1 (if blastic morphology, please add AMPO, ckit, CD10, CD19/Pax5, Tdt, CD34)
- Optimal/extended work-up :
    - IHC: Mum1, cmyc, Bcl-2, EBV-LMP1/EBER, CD43, CD138
    - FISH: CMYC/BCL2/BCL6; IFR4 gene rearrangement
    - Sequencing: MYD88 mutation

5. T-NHL-requisites for diagnosis

CD3 positive NHL: large cell morphology

- Mandatory:
    - IHC: CD20, CD3, CD30, MIB-1, ALK-1
- Optimal/extended work-up :
    - IHC: CD3, CD20, MIB-1, CD56, CD30, ALK-1, CD10, Bcl6, PD1, Mum1, EBV-LMP1/EBER, CD123, Gata3, CD4, CD8, CD2, CD7
    - FISH: DUSP22 gene rearrangement

CD3 positive NHL: non-large cell morphology

- Mandatory:
    - IHC: CD20, CD3, CD2, CD5, CD7, CD4, CD8, MIB-1, cyclin D1, Tdt, CD34, CD30, ALK-1
- Optimal/extended work-up :
    - IHC: CD56, CD10, Bcl6, PD1, Mum1, EBER-ISH, CD123, Gata3
    - FISH: DUSP22 gene rearrangement

6. CD3 and CD20 negative NHL- requisites for diagnosis

- IHC: LCA, CD3, CD20, CD30, CD19/Pax-5, CD138, ALK-1, CD5, CD10, Bcl6, Mum1, EBV-LMP1/EBER, CD56, CD7, CD4, CD8, CD123, MIB-1, c-kit, MPO, CD34, Tdt, CD1a, CD163, S-100 protein, CD23, CD21, kappa, lambda, MIB-1

<u>Important</u>: The laboratory without expertise in diagnosing hematolymphoid neoplasms and with inadequate IHC/Flow cytometric immunophenotyping panels should refer the sample to any specialized lab dealing with such neoplasms. There can't be any definite algorithms for diagnosing hematolymphoid neoplasms as each lesion is different and number of reagents used may vary case to case basis.

Annexure 3. FCM panel for hematolymphoid malignancies

1. Processing, Instrument Setup and Quality Control

Processing, Instrument Setup and Quality Control should be done as per Euroflow protocols or ICMR guidelines published in 2016. The links are given below

https://www.icmr.nic.in/sites/default/files/guidelines/Immunophenotyping%20of%20Hematolymphoid%20Neoplasms_0.pdf

https://www.euroflow.org/usr/pub/prlogin.php

2. Acute Leukemia- Essential panel

1. Smears stained with a Romanowsky stain and Myeloperoxidase or Sudan Black B
2. NSE, toluidine blue and Iron stain as required.

Note: Morphology is followed by flow cytometric immunophenotyping and other ancillary techniques including cytogenetics and molecular diagnostics. The final diagnosis is based on a combination of all these modalities.

Essential		
Common markers	CD45, CD38, HLADR	
Markers of immaturity	CD34	
	Lineage associated	Lineage Specific
B-cell	CD10, CD19, CD20, surface or cytoplasmic CD22, CyCD79a	
T-cell	CD1a, CD4, CD5, CD7, CD8, TCRγδ	Surface and Cytoplasmic CD3
Myeloid	CD13, CD33, CD117	cyMPO or Cytochemical Myeloperoxidase or Sudan Black B
Monocytic	CD36, CD64	Non Specific Esterase
Megakaryoblastic	X	
NK-cell	CD56	

Plasmacytoid dendritic cells	CD123	

3. Acute leukemia- Optimal

Essential and the following

Optimal – Essential and the following		
Common markers		
Markers of immaturity		
	Lineage associated	Lineage Specific
B-cell	CD73, CD86, CD25, CD304	
T-cell		
Myeloid	CD15,	
Monocytic	CD11c, CD14,	
Megakaryoblastic	CD41, CD61	
NK-cell		
Plasmacytoid dendritic cells		

4. **Acute leukemia- Optional**

Optimal with the following

Common markers	CD25, CD45RA	
Markers of immaturity	CD133, TdT	
	Lineage associated	**Lineage Specific**
B-cell	CD58, CD81, NG2, CRLF2	IgM, Kappa & Lambda light chains
T-cell	CD2, CD99, TCRαβ	
Myeloid	CD15, CD11b, CD16, CD65, CD66c	
Monocytic	CD86, CD300e	
Megakaryoblastic	CD42b	
NK-cell	CD94, CD161	
Plasmacytoid dendritic cells	CD303, CD304	
Mast cells	CD203c	
Erythroid lineage	CD49d, CD71, CD105	CD235a

5. DNA ploidy for B-ALL

Propidium Iodide

FxCycle Violet

DRAQ5

DAPI (4’,6-Diamidino-2-phenyl Indole)

6. B-ALL MRD

Essential	Optimal	Optional
CD10, CD19, CD20, CD34, CD38, CD45, CD73, CD123, CD86, CD304		CD25, CD44, CD66c, CD81, CD200, CD58
Nuclear dye such as Syto13, Syto16, Syto44		

Recommendations for processing

- Use Euroflow recommended Bulk-lysis method
- Acquire minimum 10,00,000 CD45-positive events
- Minimum 8-color antibody panel
- Use the template-based analysis
- Should be done in a laboratory with workload of minimum 30 acute leukemia samples per month
- Mention the limit of detection and limit of quantitation of MRD assay
- Mentioned the number of events studied
- Control sample should be evaluated atleast once in month

7. T-ALL MRD

Optimal	Optional
CD4, CD5, CD7, CD8, CD16, CD34, CD38, CD45, CD56, Surface and cytoplasmic CD3	CD1a, CD2, CD48, CD99, TdT
Nuclear dye such as Syto13, Syto16, Syto44	

8. AML MRD

	Optimal	**Optional**
Deviation from normal	CD13, CD14, CD15, CD33, CD34, CD36, CD38, CD45, CD64, CD117, CD123, HLADR	CD11b, CD65, CD66c, CD71,
Leukemia associated Immunophenotypic markers	CD7, CD19, CD56	CD2, CD4, CD5,

9. Lymphoproliferative disorders/ Lymphoma

B-cell NHL

Essential	Optimal	Optional
CD5, CD10, CD19, CD20, CD23, CD45, CD200, Kappa & Lambda light chains	CD22, CD38, IgM,	CD27, CD43, CD44, CD49d, CD72, CD79b, CD81, CD123, CD148, CD180, CD305, IgD, IgG, FMC7, ROR1, Ki67, BCL2, BCL6, Mum-1
Hairy Cell Leukemia / Splenic Lymphomas CD11c, CD25, CD103, CD123		

T- NHL

Essential	Optimal	Optional
CD3, CD4, CD5, CD7, CD8, CD10, CD16, CD25, CD26, CD45, CD56,	CD2, CD30, CD94, CD161, CD185, CD279, ALK-1, Perforin, Granzyme	CD38, CD45RA, CD45RO, TCL1, TCRVβ-repertoire, KIR, Ki67, TIA-1

10. MRD- myeloma

Optional		
Plasma cell gating markers	**Surface markers**	**Cytoplasmic markers**
CD38, CD138, CD45, CD229*, CD319*	CD19, CD20, CD45, CD27, CD28, CD56, CD81, CD117, CD200,	Kappa & Lambda light chains
* In patients treated with Daratumumab therapy		

<u>Important</u>: The laboratory without expertise in diagnosing hematolymphoid neoplasms and with inadequate IHC/Flow cytometric immunophenotyping panels should refer the sample to any specialized lab dealing with such neoplasms. There can't be any definite algorithms for diagnosing hematolymphoid neoplasms as each lesion is different and number of reagents used may vary case to case basis.