Use of Immunophenotyping/Genetic Testing in Differential Diagnosis of Mature B-Cell and NK/T-Cell Neoplasms
USE OF IMMUNOPHENOTYPING/GENETIC TESTING IN DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL AND NK/T-CELL NEOPLASMS

(To be used in conjunction with clinical and morphologic correlation)

GENERAL PRINCIPLES

• Morpohology ± clinical features drive both the choice and the interpretation of special studies.
• Differential diagnosis is based on morphology ± clinical setting.
• Begin with a broad panel appropriate to morphologic diagnosis, limiting panel of antibodies based on the differential diagnosis.
  ▶ Avoid “shotgun” panels of unnecessary antibodies unless a clinically urgent situation warrants.
• Add antigens in additional panels, based on initial results.
• Follow with genetic studies as needed.
• Return to clinical picture if immunophenotype + morphology are not specific.

Continued on next page (NHODG-A 2 of 11)
USE OF IMMUNOPHENOTYPING/GENETIC TESTING IN DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL AND NK/T-CELL NEOPLASMS

(TO BE USED IN CONJUNCTION WITH CLINICAL AND MORPHOLOGIC CORRELATION)

B-cell antigens positive (CD19, CD20, CD79a, PAX5)
- Morphology
  - Cytology
    - Small cells
    - Medium-sized cells
    - Large cells
  - Pattern
    - Diffuse
    - Nodular, follicular, mantle, marginal
    - Sinuses
- Clinical
  - Age (child, adult)
  - Location
    - Nodal
    - Extranodal, specific site
- Immunophenotype
  - Naïve B cells: CD5, CD23
  - GCB cells: CD10, BCL6, FDC (CD21, CD23)
  - Post-GCB cells: IRF4/MUM1, CD138
  - Immunoglobulin heavy and light chains (surface, cytoplasmic, class switch, light chain type)
  - Oncogene products: BCL2, cyclin D1, MYC, BCL6, ALK
  - Viruses: EBV, HHV8
  - Other: CD43, Ki-67
- Genetic testing
  - BCL2, BCL6, CCND1, MYC, ALK, MYD88, BRAF, IG rearrangement

T- or NK/T-cell antigens positive (CD2, CD3, CD5, CD7)
- Morphology
  - Anaplastic vs. non-anaplastic
  - Epidermotropic
- Clinical
  - Age (child, adult)
  - Location
    - Cutaneous
    - Extranodal noncutaneous (specific site)
    - Nodal
- Immunophenotype
  - CD30, ALK*, CD56, CD1a, TdT
  - Follicular T-cells: CD10, BCL6, CD57, CD279 (PD1)
  - Viruses: EBV, HTLV1 (clonal)
- Genetic testing
  - ALK, TCR, HTLV1

*Always do ALK if CD30+

See Initial Morphologic, Clinical, and Immunophenotypic Analysis (NHODG-A 3 of 11)

---

Note: All recommendations are category 2A unless otherwise indicated. Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
INITIAL MORPHOLOGIC, CLINICAL, AND IMMUNOPHENOTYPIC ANALYSIS

Lineage-based on immunophenotype (Pan-B and Pan-T antigens) or Suspected by morphology/clinical features

- B-cell neoplasms
  - Small cells
  - Medium-sized cells
  - Large cells ± anaplastic morphology
  - Cutaneous localization

- T-cell neoplasms
  - Anaplastic morphology
  - Cutaneous localization (non-anaplastic morphology)
  - Extranodal, noncutaneous localization (non-anaplastic morphology)
  - Nodal localization (non-anaplastic morphology)

See NHODG-A 4 of 11
See NHODG-A 5 of 11
See NHODG-A 6 of 11
See NHODG-A 8 of 11
See NHODG-A 9 of 11
See NHODG-A 10 of 11
See NHODG-A 11 of 11

See NHODG-A 11 of 11

These are meant to be general guidelines. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.

Initial panel will often include additional markers based on morphologic differential diagnosis and clinical features.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
DISCUSSION

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

Flow cytometry on blood or bone marrow done only if HCL is in differential diagnosis by morphology.

Rare cases of HCL may be CD10+ or CD5+ and some cases of FL are CD10-. BCL6 is a useful discriminate if needed (rarely). Rare cases of MCL are CD5-.

Confirmation with BRAF sequencing or IHC for mutant protein

Morphology (MZ pattern)

Clinical features (extranodal, splenic)

Pseudofollicular pattern, clinical features (BM)

Morphology (MZ pattern, plasmacytoid features), genetics (del 7q)

Clinical features (splenomegaly, BM involvement, paraprotein)

CD5+ cyclin D1+ t(11;14)+

Cyclin D1- t(11;14)- trisomy 12

del(11q) del(13q) del(17p)

MCL

FL

HCL

CD5- CD10- Cyclin D1- t(11;14)-

CD103- CD25- CD11c-

HCL

CD123+ annexin A1+

MZL

CD5+ cyclin D1+ t(11;14)+

Cyclin D1- t(11;14)-

BCL6+ BCL2+d t(14;18)+

CD103+ e CD25+ CD11c+ e

CD103- i

Cytoplasmic Ig-j

Cytoplasmic Ig+j

 Confirmation with sequencing or IHC for mutant protein

BRAF

MYD88 mut+

LPL

MYD88 mut-

MZL

aThese are meant to be general guidelines. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.

Rare cases of HCL and t(11;14) negative MCL have been reported. This diagnosis should be made with extreme caution and with expert consultation.

85% of follicular lymphoma will be BCL2+ or t(14;18)+. Kappa and lambda light chains; IgG, IgM, and IgA may be helpful.

Can be done to confirm if necessary.

a-b These are meant to be general guidelines. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.

Rare cases of cyclin D1 and t(11;14) negative MCL have been reported. This diagnosis should be made with extreme caution and with expert consultation.

85% of follicular lymphoma will be BCL2+ or t(14;18)+.

Kappa and lambda light chains; IgG, IgM, and IgA may be helpful.
B-CELL NEOPLASMS

**Diffuse pattern**
- Medium cells ± starry sky pattern

**Initial Panel:**
- CD5, CD10, cyclin D1, BCL2, BCL6, Ki-67

**Cyclin D1+**
- MCL, blastoid variant

**Cyclin D1-**
- **BCL6+/-**
  - IRF4/MUM1+/-
  - MCL, blastoid variant
  - CLL with increased prolymphocytes
  - DLBCL, NOS
  - MCL

**BCL6-**
- **BCL2+**
  - FISH for MYC, BCL2, BCL6
  - U-DLBCL/BL
  - FISH for MYC, BCL2, BCL6 to check for “double hit”

**BCL6+/BCL2-**
- Consider plasma cell neoplasm

**BCL6+/BCL2+**
- **IRF4/MUM1-**
  - FISH for MYC, BCL2, BCL6
  - U-DLBCL/BL
  - FISH for MYC, BCL2, BCL6 to check for “double hit”

**BCL6+/-**
- **BCL2+**
  - IRF4/MUM1+/-
  - U-DLBCL/BL
  - FISH for MYC, BCL2, BCL6 to check for “double hit”

**Medium cells**
- Burkitt lymphoma (BL)
- Diffuse large B-cell lymphoma (DLBCL)
- Mantle cell lymphoma (MCL), blastoid variant
- B-cell lymphoma (BCL), unclassifiable, intermediate between DLBCL and BL (U-DLBCL/BL)

**Note:**
- All recommendations are category 2A unless otherwise indicated.
- Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
USE OF IMMUNOPHENOTYPING/GENETIC TESTING IN DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL AND NK/T-CELL NEOPLASMS

(TO BE USED IN CONJUNCTION WITH CLINICAL AND MORPHOLOGIC CORRELATION)

B-CELL NEOPLASMS

Large cells:
- Diffuse large B-cell lymphoma (DLBCL), NOS
  - T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL)
  - Primary DLBCL of the CNS
  - Primary cutaneous DLBCL, leg type
  - EBV-positive DLBCL of the elderly (EBV + DLBCL)
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma (PMBL)
- Intravascular large B-cell lymphoma
- ALK-positive large B-cell lymphoma
- Plasmablastic lymphoma
- Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease (LBCL in HHV8 + MCD)
- Primary effusion lymphoma
- B-cell lymphoma, unclassifiable, intermediate between DLBCL (U-DLBCL) and classical Hodgkin lymphoma (CHL)
- Mantle cell lymphoma (MCL), pleomorphic variant

Panel: CD5, CD10, BCL6, IRF4/MUM1, Ki-67

CD5+
- Cyclin D1+ Pleomorphic MCL
  - CD5+ Cyclin D1-
    - CD10+ DLBCL, NOS CD5+
    - CD10- DLBCL
  - CD10+ DLBCL, NOS GCB type (BCL6+)
  - CD10- DLBCL, NOS GCB type

GCB= Germinal center B-cell like

Panel: CD20, PAX5, CD138, Ig light and heavy chains (If further characterization is warranted based on clinical or morphologic features. The specific panel will vary depending on the differential diagnosis.)

CD5- DLBCL

BCL6+ IRF4/MUM1-
- Non-GCB
  - BCL6+ IRF4/MUM1+ Post-GCB

CD10+ DLBCL, NOS GCB type (BCL6+)

Panel: CD20, PAX5, CD138, ALK1, CD30, CD15, EBV-EBER, HHV8, Ig light and heavy chains (If further characterization is warranted based on clinical or morphologic features. The specific panel will vary depending on the differential diagnosis.)

Panel: CD5, CD10, BCL6, IRF4/MUM1, Ki-67

CD5+
- Cyclin D1+ Pleomorphic MCL
  - CD5+ Cyclin D1-
    - CD10+ DLBCL, NOS CD5+
    - CD10- DLBCL
  - CD10+ DLBCL, NOS GCB type (BCL6+)
  - CD10- DLBCL, NOS GCB type

GCB= Germinal center B-cell like

Panel: CD20, PAX5, CD138, ALK1, CD30, CD15, EBV-EBER, HHV8, Ig light and heavy chains (If further characterization is warranted based on clinical or morphologic features. The specific panel will vary depending on the differential diagnosis.)

Note: All recommendations are category 2A unless otherwise indicated. Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

Continued on next page

These are meant to be general guidelines. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.

1Ki-67 is a prognostic factor in some lymphomas. (eg, mantle cell and is typically >90% in Burkitt lymphoma.) It is not useful in predicting the presence of MYC rearrangement or in classification.

aCD5 is included to identify pleomorphic MCL; if CD5 is positive, cyclin D1 staining is done to confirm or exclude MCL.
NCCN Guidelines Version 2.2015
Non-Hodgkin’s Lymphomas

USE OF IMMUNOPHENOTYPING/GENETIC TESTING IN DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL AND NK/T-CELL NEOPLASMS

(TO BE USED IN CONJUNCTION WITH CLINICAL AND MORPHOLOGIC CORRELATION)

**Large cells (continued)**

- **EBER-**
  - **CD30 -**
    - DLBCL, non-GCB
  - **CD30 +**
    - Mediastinal
    - **PMBL (May be BCL6+, IRF4/MUM1-)**
    - **Morphologically borderline with CHL**
      - **CD15 -**
        - **PMBL**
      - **CD15 +**
        - **U-DLBCL/CHL**
  - **Elderly or immunosuppressed**
    - **EBV + DLBCL**
  - **Extranodal, T-cell rich, angiocentric**
    - Lymphomatoid granulomatosis
  - **Chronic inflammation**
    - **DLBCL associated with chronic inflammation**

- **EBER-**
  - **HHV8+**
    - **LBCL in HHV8 + MCD (IgM lambda +) confirm by morphology**

- **CD20+ (PAX5+)**
  - **EBER+**
    - **CD30 -**
      - T-cell-rich
      - **THRLBCL (May be BCL6+, IRF4/MUM1-)**
    - **CD30 +**
      - Mediastinal
      - **PMBL (May be BCL6+, IRF4/MUM1-)**

- **CD20- (PAX5-) CD79a+ MUM1+**
  - **EBV-**
    - **HHV8-**
      - **Plasmablastic lymphoma**
        - **MYC FISH +**
    - **EBV+/- HHV8+**
      - **PEL (CD30+)**
    - **EBV-**
      - **ALK+**
        - **ALK + DBLCL**
        - **IgA lambda + EMA +**
      - **EBV-**
        - **ALK-HHV8-**
          - **Anaplastic/Plasmablastic myeloma/plasmacytoma**
          - **CD56 +/- Cyclin D1 +/- IgG, IgA, kappa, or lambda**

**Note:** All recommendations are category 2A unless otherwise indicated. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.

**Clinical Trials:** NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
**Non-Hodgkin’s Lymphomas**

**USE OF IMMUNOPHENOTYPING/GENETIC TESTING IN DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL AND NK/T-CELL NEOPLASMS**

**B-CELL NEOPLASMS**

- **Primary cutaneous marginal zone lymphoma (PCMZL)**
- **Primary cutaneous follicle center lymphoma (PCFCL)**
- **Primary cutaneous DLBCL, leg type (PC-DLBCL, leg type)**

**Panel:** CD3, CD5, CD10, BCL2, BCL6, IRF4/MUM1, CD21/23 (FDC markers)

- **Cutaneous localization**
  - **CD10+**
    - **BCL6+**
      - **IRF4/MUM1-**
        - Small/medium/large cells
        - Many CD3+ cells
      - **FDC = Follicular dendritic cells**
    - **BCL6-** (positive GC)
      - **IRF4/MUM1+/-**
        - Follicular, disrupted
      - Small/medium cells
      - Larger cells in GC
  - **CD10-**
    - **BCL2-**
      - **BCL6+**
        - **IRF4/MUM1-**
          - FDC+/-
          - Small/medium/large cells
          - Many CD3+ cells
      - **BCL2-** (positive GC)
        - **IRF4/MUM1+/-**
          - Follicular, disrupted
        - Small/medium cells
        - Larger cells in GC
    - **BCL2 strongly +**
      - **BCL6+/-**
        - IRF4/MUM1+
        - FDC -
      - Large round cells
      - Few CD3+ T-cells
  - **BCL2+**
    - **BCL6+**
      - **IRF4/MUM1-**
        - FDC±, follicular
        - Small/medium/large cells
        - Many CD3+ T-cells
      - **PCFCL**
    - **BCL6+**
      - **IRF4/MUM1-**
        - FDC±, follicular
        - Small/medium/large cells
        - Many CD3+ T-cells
      - **PCMZL**
    - **BCL6-** (positive GC)
      - **IRF4/MUM1+/-**
        - Follicular, disrupted
      - Small/medium cells
      - Larger cells in GC
      - **PC-DBLCL, leg type**
    - **PCFCL**

**Note:** All recommendations are category 2A unless otherwise indicated. Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

---

*aThese are meant to be general guidelines. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.

*These are assessed both in follicles (if present) and in intrafollicular/diffuse areas. CD10+ BCL6+ germinal centers are present in PCMZL, while both follicular and interfollicular/diffuse areas (tumor cells) are positive for BCL6+/- CD10 in PCFCL.*
USE OF IMMUNOPHENOTYPING/GENETIC TESTING IN DIFFERENTIAL DIAGNOSIS OF MATURE B-CELL AND NK/T-CELL NEOPLASMS

(TO BE USED IN CONJUNCTION WITH CLINICAL AND MORPHOLOGIC CORRELATION)

T-CELL NEOPLASMS

Anaplastic morphology

- Anaplastic large cell lymphoma (ALCL), ALK positive
- Anaplastic large cell lymphoma (ALCL), ALK negative
- Adult T-cell leukemia/lymphoma (ATLL), anaplastic large cell type
- Enteropathy associated T-cell lymphoma (EATL)
- Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
  - Lymphomatoid papulosis (LyP)
  - Primary cutaneous anaplastic large cell lymphoma (PC-ALCL)

Panel: CD30, CD15, PAX5, ALK, EBV-EBER, cytotoxic granule proteins (granzyme B, perforin, TIA1), CD25, MUM1

- Anaplastic morphology
  - CD30+ strong, all cells
  - PAX5+
  - ALK+ → ALCL, ALK+
  - PAX5 Dim+
  - CD15+/EBER+-→ Consider CHL (T-cell antigen expression may rarely occur in CHL)
  - ALK- → PAX5+-→ If only one T-cell antigen expressed, could be DLBCL
  - CD30 or focal → PTCL-NOS

- Cutaneous = Primary cutaneous CD30+ T-cell LPD
  - Polymorphous, regressing = LyP
  - Monomorphous, progressing = PC-ALCL
  - MF in transformation (if history of MF)
- Non-cutaneous = ALCL, ALK- (caveat: rule out nodal involvement by CTCL, CD15 maybe + in CTCL)
- Intestinal = EATL (eosinophils: clinical history of celiac disease or antibodies)
- HTLV1+ = ATLL, anaplastic large cell type (CD25+)

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
Non-Hodgkin’s Lymphomas

T-CELL NEOPLASMS

**Cutaneous localization (non-anaplastic morphology)**

- Primary cutaneous CD30-positive T-cell lymphoproliferative disorders (LPD)
- Mycosis fungoides, Sézary syndrome (MF, SS)
- Subcutaneous panniculitis-like T-cell lymphoma (SCPTCL)
- Primary cutaneous gamma-delta T-cell lymphoma (γδTCL)
- Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma (AECTCL)
- Primary cutaneous CD4-positive small/medium T-cell lymphoma
- Extracutaneous NK/T-cell lymphoma, nasal type
- Peripheral T-cell lymphoma, NOS
- Blastic plasmacytoid dendritic cell (BPDC) neoplasm

**Cutaneous localization (non-anaplastic morphology)**

- Mycosis fungoides, Sézary syndrome (MF, SS)
- Subcutaneous panniculitis-like T-cell lymphoma (SCPTCL)
- Primary cutaneous gamma-delta T-cell lymphoma (γδTCL)
- Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma (AECTCL)
- Primary cutaneous CD4-positive small/medium T-cell lymphoma
- Extranodal NK/T-cell lymphoma, nasal type
- Peripheral T-cell lymphoma, NOS
- Blastic plasmacytoid dendritic cell (BPDC) neoplasm

**Panel:** CD2, CD5, CD7, CD4, CD8, CD30, CD56, βF1, TCRγ, cytotoxic granule proteins (CGP = perforin, granzyme B, TIA1), EBV-EBER; Optional: CD25, CD279

**CD30+ strong, all cells**

- MF, SS (CD2+ CD5+ CD7- CD8- βF1+ CGP-)
- HTLV1 + = ATLL

**CD30-or focal**

**Epidermotropic**

- CD4-
- CD4+

**CD4+**

- CD8+
- CD8-

**CD8+**

- βF1+
- βF1-

**CD8-**

- EBV+
- EBV-

**CD56+**

- SCPTCL (CD2+ CD5- CD7+ CD56+ CGP+)
- Cutaneous γδTCL (CD2+ CD5- CD7+/- CD56+/- CGP+)
- PTCL-NOS

**CD56-**

- ENK/TL nasal type (CD2+ CD7- CD56+ CGP+, TCRγ-)
- Cutaneous γδTCL (CD2+ CD5- CD7+/- CD56+/- CGP+, TCRγ+)

**Consider myeloid sarcoma (may be CD2+ CD7+ CD56+) or BPDC (CD3- CD5- CD123+ CD68+ TCL1+)**

**Small/med cells = CD4+ small/medium CTCL/ T-cell pseudolymphoma (CD279+) Med/ large cells = PTCL, NOS**

**γδ**

- SCPTCL (CD2+ CD5- CD7+ CD56+ CGP+)
- Cutaneous γδTCL (CD2+ CD5- CD7+/- CD56+/- CGP+)
- PTCL-NOS

**βF1+**

- ENK/TL nasal type (CD2+ CD7- CD56+ CGP+, TCRγ-)
- Cutaneous γδTCL (CD2+ CD5- CD7+/- CD56+/- CGP+, TCRγ+)

Note: All recommendations are category 2A unless otherwise indicated. Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
Extranodal, noncutaneous localization (non-anaplastic morphology)

- Extranodal NK/T cell lymphoma, nasal type (ENKTCL)
- Enteropathy-associated T-cell lymphoma (EATL)
- Hepatosplenic T-cell lymphoma (HSTCL)
- Peripheral T-cell lymphoma, NOS (PTCL, NOS)
- ALCL, ALK+ small cell and histiocytic-rich variants

Panel: CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD56, ALK1, βF1, TCRγ, MUM1, cytotoxic granule proteins (CGP = perforin, granzyme B, TIA1), EBV-EBER

Extranodal, noncutaneous localization

- ENKTCL (CD5- CD4- CD8- CD30- CD56+ CGP+, midline face, upper aerodigestive tract, testis, GI tract) (may have T-cell phenotype)
  - EBER+
  - CD30-

- ALK+
  - ALCL, ALK+ small cell or histiocytic-rich variants
  - Intestinal, other abdominal/visceral sites, celiac disease or markers positive = EATL Type 1 (CD5- CD7- CD4- CD8+/- CD56+/- TIA1+ GRB+ Perf+)
  - Other sites, celiac disease markers negative = PTCL, NOS (usually less strongly CD30+)

- EBER-
  - CD30+

Nodal localization (non-anaplastic morphology)

Panel: CD2, CD3, CD4, CD5, CD7, CD8, CD30, ALK1, CD10, BCL6, PD1/CD279, CXCL 13, CD21, CD23, EBV-EBER

Nodal localization

- Adult T-cell leukemia/lymphoma (ATLL)
- Angioimmunoblastic T-cell lymphoma (AITL)
- Peripheral T-cell lymphoma, NOS (PTCL, NOS)
- ALCL, ALK+ small cell and histiocytic-rich variants

Panel: CD4, CD8, CD30, ALK1, CD10, BCL6, PD1/CD279, CXCL 13, CD21, CD23, EBV-EBER

- Vascular proliferation, expanded CD21+ CD23+ FDC = AITL
- Nodular CD21+ CD23+ FDC = Follicular PTCL

- HTLV1+ = ATLL (CD2+ CD5+ CD7- CD25+ CD56-)
- HTLV1- = PTCL, NOS

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

These are meant to be general guidelines. Interpretation of results should be based on individual circumstances and may vary. Not all tests will be required in every case.